The Effect of Hydroxyurea on Erythropoiesis, Erythrocyte Survival, and Erythrokinetics in the Rabbit

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SUMMARY

In a study of the effect of the administration of hydroxyurea in doses of 100–200 mg/kg/day on erythropoiesis, erythrocyte survival, and erythrokinetics, 10 of 12 rabbits developed a normocytic anemia, a decrease in plasma iron transport rate, and a reduction in red cell iron uptake, but no significant change in erythrocyte survival. The anemia was reversible and prompt recovery occurred when the drug was stopped. In 2 animals the anemia was associated with a moderate shortening of erythrocyte survival without a reduction in the plasma iron transport rate or the red cell iron uptake. In animals with phenylhydrazine-induced hemolytic anemia, hydroxyurea in a dose of 200 mg/kg/day impaired erythropoiesis, but it did not block the production of macrocytic erythrocytes in response to the erythropoietic stress.

INTRODUCTION

Hydroxyurea (Hydrea, E. R. Squibb and Sons Limited, Montreal 3, Quebec) has been used in the treatment of a variety of malignant diseases (2, 3, 11–13, 16, 18), and the most encouraging results to date have been obtained in chronic myelogenous leukemia (11, 12, 16). The effectiveness of hydroxyurea in the treatment of this disorder is due to the depression of the bone marrow and the resultant decrease in granulocyte production. In therapeutic doses it also produces both depression of erythropoiesis (4, 11, 13, 17) and hemolysis (11), and the development of anemia has limited the usefulness of the drug.

The results of several studies in experimental animals have shown that the administration of hydroxyurea produces a progressive anemia (1, 14, 15), but the mechanism of the anemia has not been fully elucidated. In the rabbit and dog Rosenthal et al. (15) found that the administration of high doses of hydroxyurea led to the production of large erythrocytes, and they believed the animals had developed a condition similar to pernicious anemia. In contrast to these results, Adamson et al. (1) found a reduction in the mean cell volume of erythrocytes in monkeys treated with hydroxyurea, and Lerner et al. (14) observed that hydroxyurea produced a hypoplastic anemia in the rat with a reduction in the mean cell volume from 64 cu μ to 60 cu μ.

The present investigation was undertaken in the rabbit to define precisely the effect of hydroxyurea on erythropoiesis, erythrocyte survival, and erythrokinetics.

MATERIALS AND METHODS

Animals

New Zealand white rabbits of both sexes, weighing between 2 kg and 3 kg, were used.

Blood Sampling

Small samples of blood were taken from the marginal ear vein with a 25-gauge needle attached to a 1-ml syringe, and large volumes were obtained by cardiac puncture. The blood was mixed with dried heparin to prevent coagulation.

Hematologic Determinations

Hematologic tests were performed by methods mentioned previously (6).

Counting of Radioactivity

A sample volume of 0.2 ml diluted in 3 ml of water was counted in a well-type scintillation counter. Where samples contained both 51Cr and 59Fe, differential counting was employed, and the calculations described by Weinstein and Beutler (19) were used to obtain the results for the individual isotopes.

Blood Volume

Blood volume was measured by a modification of the Evans blue technic (10). Evans blue (Fisher Scientific Company, Montreal) was injected in a dose of 5 mg/kg as a 0.5% solution into the marginal ear vein of the rabbit. Blood samples of 0.5 ml were taken from the opposite ear at 15, 30, and 60 minutes, and the concentration of the dye in 0.2 ml of plasma was measured spectrophotometrically at 610 μμ. The blood volume was calculated from the plasma volume using a value of 0.81 for the conversion of the venous hematocrit to the whole-body hematocrit. This conversion factor was derived from a preliminary experiment in which simultaneous determinations of the Evans blue plasma volume and the 51Cr red cell mass were made in ten rabbits.
Red Cell Survival

The half-life of the red cells was measured by a modification of the $^{51}$Cr technic (7). Ten ml of blood removed by cardiac puncture were added to 1.0 ml of acid: citrate: dextrose solution (Abbott Laboratories, North Chicago, Illinois) and then incubated for 30 minutes with $60 \mu$C of $^{51}$Cr in the form of sodium chromate, approximate specific activity 183 $\mu$C/mg (Abbott Laboratories). One hundred mg of ascorbic acid were added, and 10 minutes later the blood was centrifuged at 2400 rpm for 30 minutes and the supernatant was discarded. The cells, washed with 6 ml of normal saline, were resuspended in 3 ml of normal saline and then injected into the marginal ear vein of the rabbit.

Blood samples of 0.2 ml were taken from the animals at 24 hours and three times weekly thereafter for the determination of radioactivity. The blood volume of the animals was measured with Evans Blue dye at weekly intervals.

To evaluate the survival of the labeled cells, the radioactivity in the total blood volume was calculated at intervals and corrections made for changes that occurred in the blood volume during the experiment. The results expressed as a percent of the value obtained 24 hours after injection of labeled cells were plotted on semilog paper and the half-life was estimated in days.

Ferrokinetics

The plasma iron transport rate (PITR) and the erythrocyte iron uptake were determined by a modification of the method of Giblett et al. (9). The plasma iron was determined as described previously (5).

Ten ml of blood were removed by sterile cardiac puncture into a tube containing 4 mg of heparin. The blood was centrifuged at 2000 rpm for 20 minutes, and 3 ml of the supernatant plasma were incubated for 20 minutes with 10 $\mu$C of $^{59}$Fe in the form of ferrous citrate solution in saline, approximate specific activity 20 $\mu$C/ug (Abbott Laboratories, North Chicago, Illinois). One-fifth ml of plasma was taken for a standard, and the remainder was injected into the marginal ear vein of the rabbit. Blood samples of 0.5 ml were removed from the opposite ear at 15, 30, and 60 minutes, and the radioactivity in 0.2 ml of plasma was determined. The $t_1$ of the injected $^{59}$Fe was determined from the results, and the PITR was then calculated and expressed as mg of iron per 100 ml of blood per 24 hours.

Samples of 0.2 ml of blood were taken at 24, 48, and 144 hours, and the erythrocyte iron uptake was calculated and expressed as a percent of the injected dose.

Preparation of Drugs

Hydroxyurea prepared as a 5.0% solution in saline immediately prior to use was injected intraperitoneally. Phenylhydrazine (PHZ) prepared as a 2.5% solution in water was given subcutaneously.

Experimental Design

Six separate groups of animals were studied: (a) Eight rabbits in a control group were given intraperitoneal injections of normal saline in a volume equivalent to that used for the injections of hydroxyurea. (b) Four animals were given daily injections of hydroxyurea in a dose of 100 mg/kg/day. (c) Eight animals were given daily injections of hydroxyurea in a dose of 200 mg/kg/day. (d) Four animals were given daily injections of PHZ in a dose of 8 mg/kg for 4 days. (e) Three animals were given daily injections of hydroxyurea, 200 mg/kg plus 4 injections of PHZ, 4 mg/kg beginning on the second day of the hydroxyurea treatment. (f) Four animals were given 11 daily injections of hydroxyurea, and the hematologic response to cessation of treatment was observed.

Hematologic values were determined prior to the start of each experiment and on the eighth day of treatment. The measurement of erythrocyte survival was started 24 hours before treatment, and the ferrokinetic studies were performed on the sixth day. Bone marrow was aspirated from the medial aspect of the proximal tibia in several of the animals during the course of treatment, and smears were stained with Wright's and Giemsa stains.

RESULTS

Hematologic Values

The mean and range of values for the hematocrit, red cell count, mean cell volume, and reticulocyte count are given in Table 1.

Control Animals. After 8 days of treatment with normal saline no significant difference occurred in the hematologic values.

Hydroxyurea (100 mg/kg/day)-treated Animals. In 3 of the 4 animals given hydroxyurea, the average hematocrit declined from 37.2% to 27.3% after 8 days of treatment. The mean cell volume and the reticulocyte concentration were unchanged from the values observed before treatment. In one animal with a reticulocyte count above the normal range prior to treatment there was a decline in the hematocrit which was accompanied by an increase in both mean cell volume and the reticulocyte concentration. This animal, unlike the other three, had shortened erythrocyte survival.

Hydroxyurea (200 mg/kg/day)-treated Animals. In 7 of 8 animals given hydroxyurea, the average hematocrit declined 40.3% to 31.1% after 8 days of treatment, and reticulocyte count fell from an average value of 1.4 to 0.3%. The mean cell volume of the erythrocytes remained unchanged. In the other animals the hematocrit fell more quickly, and the reticulocyte count remained unchanged. This rabbit, unlike the other 7, had a shortened red cell life span.

Phenylhydrazine-treated Animals. The response to PHZ induced hemolysis was the production of large macrocytic erythrocytes with an average mean cell volume of 120 cu $\mu$, which is approximately twice normal.

Hydroxyurea: Phenylhydrazine-treated Animals. When hemolysis was produced during the course of treatment with hydroxyurea, there was a marked decline in the hematocrit and a marked increase in the reticulocyte concentration from 2.3 to 19.4%. The erythrocytes produced in response to the hemolysis were much larger than normal; the mean cell volume of the erythrocytes was 91.2 cu $\mu$.
Response to Cessation of Hydroxyurea Treatment. Cessation of treatment after 11 daily injections of hydroxyurea was followed by a marked reticulocytosis, about a 10% increase in the mean cell volume, and a progressive rise in the hematocrit (Chart 1).

Erythrocyte Survival

The mean and range of values for the $t_4$ of the $^{51}$Cr-labeled red cells are given in Table 1. The average $t_4$ of the red cells in the control group, 11.3 days, was not significantly different from the value of 13.5 days in 3 of the 4 animals given hydroxyurea in the dose of 100 mg/kg/day or from the value 12.7 days in 7 of 8 animals given hydroxyurea in the dose of 200 mg/kg/day. In two of the animals, one given the smaller and the other the larger dose of hydroxyurea, the erythrocyte half-life was shortened to six days. It should be noted that the reticulocyte count was slightly increased in both of these animals prior to treatment.

Blood Volume

The values for the total blood volume and red cell mass in the various groups of animals 6 days following the onset of treatment are given in Table 2. The blood volume was increased in the animals treated with a combination of hydroxyurea and phenylhydrazine; otherwise no significant difference was observed among the various groups.
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Table 2

<table>
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<tr>
<th>Treatment (mg/kg/day)</th>
<th>Number of animals</th>
<th>Plasma iron (μg/100 ml)</th>
<th>αFe t1/2 (min)</th>
<th>PITR (mg/100 ml/24 hours)</th>
<th>Red cell iron uptake (%)</th>
<th>Red cell mass (ml/kg)</th>
<th>Blood volume (ml/kg)</th>
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Ferrokinetics and red cell mass on the sixth day of treatment (mean value and range). PITR, plasma iron transport rate.

* Animals in which hemolysis occurred.

Ferrokinetics

The results of the PITR and erythrocyte iron uptake are given in Table 2.

**Control Animals.** The average PITR was 2.9 mg/100 ml, and the average erythrocyte iron uptake at 48 hours was 78%.

**Hydroxyurea (100 mg/kg/day)-treated Animals.** In 3 of the 4 animals, treatment led to a slight reduction in the PITR to 2.3 mg/100 ml and a moderate decrease in the erythrocyte iron uptake to 44% in 48 hours. In the animals in which the response to hydroxyurea was a shortening of the red cell survival, the PITR and erythrocyte iron uptake were raised to the upper limit of normal.

**Hydroxyurea (200 mg/kg/day)-treated Animals.** In 7 of the 8 animals, treatment led to a significant decline in the PITR to 1.6 mg/100 ml and to a decrease in the erythrocyte iron uptake to 24% in 48 hours. In the animal in which the response to hydroxyurea was a shortening of the red cell survival, the PITR and erythrocyte iron uptake were within normal limits.

**PHZ-treated Animals.** In response to hemolysis, there was a significant increase in both the PITR and erythrocyte iron uptake to values of 5.9 mg/100 ml/24 hours and 100% at 48 hours respectively.

**Hydroxyurea-Phenylhydrazine-treated Animals.** The PITR was within the normal range, but the erythrocyte iron uptake was significantly reduced.

Bone Marrow Morphology

The stained smears of bone marrow aspirates from the animals given 200 mg/kg of hydroxyurea showed normoblastic erythropoiesis and moderate hypoplasia of the erythroid elements. No megaloblastic changes were observed.

DISCUSSION

It is evident from the results of the present study in the rabbit that hydroxyurea in a dose of either 100 or 200 mg/kg/day produces depression of erythropoiesis, as evidenced by a decline in both the PITR and the red cell iron uptake and a decrease in the red cell mass. In addition, at the higher dose there is a reduction in the reticulocytes in the peripheral blood and erythropoiesis of the bone marrow. The ferrokinetic abnormalities are similar to those reported by Alfrey et al. (3) in patients treated with hydroxyurea, but the normoblastic erythropoiesis differs from the megaloblastosis seen in patients given similar doses of hydroxyurea on a weight for weight basis (4, 17, 18). The diminution in erythropoiesis is probably due to impaired DNA synthesis (8, 11), with a consequent diminution in cellular proliferation in the marrow. It is evident that this effect is short lived with the doses that were employed in this study because prompt recovery occurred when the drug was stopped (Chart 1).

There were two animals at each dose of hydroxyurea that had a 50% reduction in erythrocyte survival. With the higher dose, 200 mg/kg/day, the PITR and red cell iron uptake in these animals were within the normal range, but with the smaller dose both of these parameters were increased. In contrast to the others, both animals had reticulocyte counts above the normal range prior to treatment. This raises the possibility that a mild hemolytic process was present before hydroxyurea was given and it was accentuated by the treatment.

The development of hemolysis during the course of hydroxyurea therapy has been reported in 2 of 9 patients with chronic myelogenous leukemia (11), but it is not clear whether this was due solely to the effect of the drug, to the disease process, or to a combination of the two factors.

In 3 animals given hydroxyurea in a dose of 200 mg/kg/day, the erythropoietic response to phenylhydrazine-induced hemolysis was the production of large macrocytic erythrocytes. The response was similar to that observed with phenylhydrazine given alone, except the PITR and red cell iron uptake were reduced and a progressive fatal anemia ensued. The results show that hydroxyurea-induced inhibition of erythropoiesis does not block the production of macrocytic erythrocytes in response to severe erythropoietic stress.

The finding of an anemia of the normochromic normocytic type with no alteration in the mean cell volume of the erythro-
cytes differs from observations of Rosenthal et al. (15), who reported macrocytic anemia in rabbits given hydroxyurea in doses 300–1000 mg/kg/day. The rapid development of anemia in their experiments indicates that hemolysis was a prominent feature with these doses, and it is very likely that the macrocytosis observed in the peripheral blood was due to large macrocytic erythrocytes produced in response to severe erythropoietic stress (6). This possibility is strengthened by the finding of mild hemolysis in 2 of our animals given much smaller doses and the observation that hydroxyurea impairs but does not inhibit the production of large macrocytic erythrocytes in response to hemolysis induced by the administration of phenylhydrazine (Table 1).

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