Hematopoiesis and Serum Iron Changes Following Vincristine Sulfate

By Mark E. Nesbit, Jr., and James T. Lowman

VINCRISTINE SULFATE, a dimeric alkaloid, is a potent inhibitor of cell division. It shares with other plant alkaloids, Vinblastine (Vbl) and Colchicine, the ability to produce a spectrum of cytologic effects on cells during metaphase. This metaphase-arresting action has been demonstrated after treatment with Vincristine Sulfate (Vcr) on cell lines in vitro, and in normal and malignant cell lines of animals and humans. The finding of an unexplained normoblastemia in the peripheral blood six or seven days following the intravenous administration of Vincristine Sulfate in children being treated for lymphatic leukemia led us to study the specific effect of Vincristine Sulfate on hematopoiesis in an animal model. The rabbit was chosen, since its size allows sequential studies of marrow and peripheral blood without sacrificing the animal. The data presented documents the selective action of moderate concentrations of Vincristine Sulfate (0.1 mg/Kg.) on marrow erythropoiesis as represented by reproducible changes in the serum iron values and peripheral and marrow red cell populations. The temporal changes produced by Vincristine Sulfate are as follows: rabbits, given a single intravenous injection of Vincristine Sulfate (0.1 mg/Kg.) develop a severe erythroid hypoplasia of their marrow by two days (48 hours). Subsequent marrow samples reveal rapid regeneration of the erythroid elements to a hyperplastic state by day six (144 hours). The serum irons and per cent saturation of the iron binding proteins reflect these changes of erythropoietic underproduction and then overproduction. Experiments with consecutive Vincristine Sulfate injections, in the same animal, reproduces these same morphologic and serum iron findings. The specific hematopoietic and serum iron changes produced by this drug in rabbits offers a model for study of both erythroid hypoplasia and hyperplasia, without appreciably effecting the other cellular elements of the marrow.

Materials and Methods

Animals

Female, white rabbits weighing 3 to 5 Kg. were used throughout the study. The animals were individually caged and observed for illnesses and mites before being used experimentally. Standard laboratory rations and water were supplied ad libitum.

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Treatment Groups

Vincristine Sulfate was intravenously administered to the following three groups:

1. Sixteen rabbits were given Vincristine Sulfate at 0.1 mg/Kg. as a single injection. Bone marrow and blood samples were obtained at 1, 3, 7, 28, 48, 72, 96, 120, 140, 168, and 192 hours.

2. Four rabbits were given Vincristine Sulfate at 0.1 mg/Kg. as a single injection. No blood samples were obtained and at 48, 72, 96, and 144 hours one animal was sacrificed and material obtained from the femurs for bone marrow sections.

3. Nine rabbits were given Vincristine Sulfate at 0.1 mg/Kg. every six days for five doses. They were followed with marrow and blood samples at 0, 3, 6, 9, 12, 15, 18, 21, 24, and 27 days.

Control Groups

Three groups of animals were followed similarly in time to Groups 1, 2, and 3 above. There were eight animals in the control group 1 for the single injection study, four animals (Group 2) were used for controls to obtain marrow sections, and seven animals were used in the control group 3 for the intermittent study.

Marrow Studies

Bone marrow aspirations were performed with specially manufactured needles that allowed direct sampling of the rabbit marrow. The hind leg (tibias and femurs) were used in rotation for the marrow samples. Direct smears were stained with Wright’s stain and a 500 cell differential was performed on four different slides from each marrow aspiration. Marrow sections were obtained by splitting the femur or tibia and fixing small sections of the marrow in Zenker’s formal. The sections were dehydrated, mounted in paraffin, sectioned and stained with hematoxalin-eosin.

Peripheral Blood Studies

Blood samples were obtained by cardiac puncture for studies of the peripheral blood elements and serum iron determinations. Hemoglobin concentrations were determined photocolorimetrically as cyanmethemoglobin, and expressed as grams per cent. White blood cell counts were made by the direct chamber method. A 100 cell differential white blood cell count was performed on blood films stained with Wright’s stain. Platelet counts were enumerated by the Rees-Ecker method and counted in the phase microscope. Reticulocytes were stained with methylene blue and counted using the Miller disc. Serum iron binding capacity was determined by the method of Schade et al.7

RESULTS

The observed effects of a single (Table 1) and repeated (Table 2) intravenous injections of Vincristine Sulfate on the red cell mass of the peripheral blood, bone marrow and serum iron values are summarized for both the rabbits in the treatment and in the control groups. The results for the observations in each category will be related separately.

Marrow Effects

Normoblasts. A single intravenous injection of Vincristine Sulfate (0.1 mg./Kg.) resulted in a selective depression of the erythroid series. This is shown in
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**Table 1: Single Injection of Vincristine Sulfate**

**Bone Marrow**

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Figure 1. The earliest significant decrease occurred at 7 hours, the percentage of erythroid elements dropped to 26 per cent from an original value of 42 per cent. The maximal depression occurred at 48 hours when the mean value for the erythroid element was 7 per cent, as compared to 46 per cent in the control animals. Following this profound erythroid hypoplasia there occurred a regeneration. Beginning at 96 hours, a hyperplastic state (69 per cent) then occurs which is seen during the remaining hours of the study.

Repeated injections of Vcr (0.1 mg./Kg. every 6 days × 5) resulted in a cyclic hypoplastic and hyperplastic state of the erythroid elements (Fig. 2). The cycle of hypoplasia followed by rebound hyperplasia was similar to that produced by a single injection. The extent of the reduction in the erythroid compartment appeared to lessen after successive injections. By day 21 and day 27 the percentage of normoblastic cells counted in the marrow three days after Vcr administration was 24 per cent and 46 per cent, respectively.

The predominant morphologic change associated with the erythroid depression was metaphase arrest. This effect was seen as early as three hours and the number of cells seen in metaphase increased to the seventh hour when 30 per cent of all distinguishable normoblastic elements were found in some form of arrest. The various patterns seen for these cytologic effects are depicted in Figure 3. The ball metaphase was the most commonly seen (Fig. 3A). Exploded chromosomal patterns are occasionally seen as shown in Figure 3B. Besides
It was observed that these obvious cytologic abnormalities of mitotic arrest, many bare nuclei, and nuclei being extruded from the cell itself, were seen throughout the marrow specimens. During the later period of maximum regeneration, the erythroid cells returned in a normal pattern and distribution. Binucleated red blood cell precursors were occasionally seen.

Cellularity. Samples taken for marrow sections at the time of maximum erythroid depression (48 hours) and erythroid regeneration (144 hours) confirmed the selective erythroid depression and subsequent regeneration suggested by the data from the aspirated marrows. The total cellularity did not change appreciably throughout these studies (Fig. 4).

Fig. 2.—Effect of repeated injections of Vcr on per cent of marrow normoblasts (9 rabbits).

Fig. 3.—Cytologic appearance of metaphase arrested normoblasts induced by Vincristine Sulfate. (A) Ball metaphase; (B) Exploded chromosomal pattern.
Granulocytopoiesis, lymphocytopoiesis. The granulocytes and lymphocytes changed in per cent as a reflection of the changes in the normoblasts. The number of these two cell types appears to remain unchanged throughout the study as determined by an estimation of these cells from a review of the bone marrow sections. No cytologic abnormalities of these cells was seen except in the marrows done after 120 hours. In these specimens there was an occasional chromosomal arrest of the granulocytic series.

Megakaryocytopoiesis. The estimated number of megakaryocytes appeared normal throughout all specimens in both the control and treated groups.

Peripheral Blood Effects

Hemoglobin, reticulocytes, and peripheral normoblasts. Figure 5A summarizes the hematologic changes seen in peripheral blood after the Vincristine
Sulfate. Control animals are shown for comparison. In the group treated with a single injection of Vincristine Sulfate, the mean hemoglobin value fell to a low of 7 Gm. per cent at 120 hours due to the combined effect of removing blood for studies and marrow depletion of the erythroid precursors (Fig. 5A). The hemoglobin value in the control animals fell to a low value of 8.2 Gm. per cent at 144 hours. Their drop is attributed only to the blood drawn for the various tests. The drop in hemoglobin in the treated animals is preceded by a drop in the reticulocyte count (0.3 per cent at 72 hours). In contrast, the reticulocyte count in control animals was 6.1 per cent at 72 hours.

Following this peripheral depression in the treated animals and commensurate with the rebound in the marrow erythroid element, there occurs a tremendous peripheral normoblastemia. The maximum value seen was 77 normoblasts per 100 white cells at 120 hours.

When the Vincristine was given repeatedly (0.1 mg./Kg. every 6 days × 5) the hemoglobin value fell slowly over the month to a low of 7.3 Gm. per cent at 27 days (Table 2). The hemoglobin in the control group at 27 days was 10.5 Gm. per cent. The rebound phenomenon, as reflected in the peripheral blood normoblasts, was again demonstrated six days after each injection of Vincristine Sulfate (Fig. 5B). There was no significant change in the other peripheral elements in either the treated or the control groups.

Granulocytes, lymphocytes, and platelets. Early in the study peripheral granulocytes, lymphocytes and platelets did not significantly differ in the treated compared to the control groups. The total white blood count from 144 to
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Fig. 6.—Serum iron and per cent normoblasts after repeated intravenous injections of Ver.

192 hours in the treated group was lower than in the control group. There are not enough animals in these later groups to determine the statistical significance of these observations.

**Serum Iron Studies**

Both the single intravenous injection of Vincristine Sulfate (0.1 mg./Kg.) and repeated intravenous injections of Vincristine Sulfate (0.1 mg./Kg. every 6 days × 5) produce an elevation of serum iron binding to almost complete saturation (Tables 1 and 2). The elevation of the serum iron and saturation of binding capacity occur just prior to the maximum depression of erythroid elements in the marrow. The fall in the serum iron and concomitant rise in the unsaturated binding capacity correlates with the rebound erythropoiesis seen following the hypoplastic marrow state. These sequentially related effects can best be seen in the study with repeated injections of Vincristine Sulfate (Fig. 6). The serum irons and binding capacities for the control animals reveal a steady fall in the iron values and a rise in the binding capacity as a result of the blood drawing.

**DISCUSSION**

The arrest of erythropoiesis by other chemotherapeutic agents such as nitrogen mustard, Cytoxan, and 5-Flourouracil in both animals and man has been well documented. In most of these cases all the marrow elements were
depressed though frequently the erythroid depression was the most markedly involved. In this report Vincristine Sulfate at a dose of 0.1 mg./Kg. (well below the LD-10 for rabbits) produces under the experimental design of these experiments a selective marrow erythroid hypoplasia. This is maximum at 48 hours. The sequential changes produced by this drug occur in two phases. The first phase is one of erythroid hypoplasia and saturation of iron-binding capacity. The second phase is an erythroid hyperplasia with a reversal of the iron data to low serum iron values and an unsaturated binding protein. The sequential changes seen during the erythroid hypoplastic state are in sequence: 1) a metaphase arrest of erythrocyte precursors beginning at 3 hours; 2) a significant fall in the percentage of erythrocyte precursors at the time of maximum metaphase arrest—7 hours; 3) a rise in the serum iron in saturation of its binding capacity by 28 hours; and 4) by 48 hours a fall in the peripheral reticulocyte count concomitant with the maximal depression of the erythroid elements and saturation of the serum iron binding capacity.

The second phase of erythroid hyperplasia is as follows: 1) a rise in the percentage of erythroid precursors within the marrow and peripheral normoblasts at 72 hours; 2) a drop in the serum iron and binding protein saturation by 96 hours; 3) peripheral reticulocytosis by 120 hours concomitant with the maximum increase in marrow normoblasts and peripheral normoblasts, and further drop in the serum iron values; and finally, 4) by 192 hours a significant rise in the peripheral hemoglobin associated with a maximum reticulocytosis.

The other elements of the marrow, including the granulopoietic, lymphocytic, and megakaryocytic elements do not appear to have been significantly affected by this dose of Vincristine Sulfate. Substantiation of this selective effect on the erythroid elements is confirmed by reviewing the marrow sections taken at the point of maximum erythroid depression and regeneration. In the rabbits treated with repeated injections of Vincristine Sulfate a portion of the erythroid precursor pool may have become resistant to its effect. After the fourth and fifth injections the percentage of erythroid precursors occurring three days post injection was 24 per cent and 46 per cent, respectively. The fact that the serum iron values repeatedly rise on days 21 and 27 suggests that there was still a significant depression of erythroid activity in these animals.

Other investigators have reported the effect of Vincristine Sulfate on hematopoiesis. Cardinali studied the effect of Vincristine Sulfate in both mice and Syrian hamsters. Stohlman studied it in normal and hypertransfused rats. Both investigators also demonstrated the metaphase arresting effect of this alkaloid on the marrow cells. Stohlman did further studies in the hypertransfused erythropoietin stimulated rat, suggesting that the effect of Vincristine Sulfate was confined entirely to the dividing cell compartment and that the precursor cell for this erythroid compartment was not affected by Vincristine. The kinetic data seen in the rapid regeneration of the erythroid elements after marrow hypoplasia in our study would support this contention. The erythroid depression in both studies appeared to be associated also with a significant depression of the myeloid series. The more selective depression of the erythroid element in our studies is most likely related to the lower dose of Vincristine Sulfate used. In the studies of Cardinali and Stohlman, the dosage...
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of Vincristine Sulfate was just below the lethal dosage for each animal population. Therefore, rather than confining the effect of Vincristine Sulfate to just mitotic inhibition of proliferating cells, these doses of Vincristine Sulfate may have caused both mitotic inhibition and overall depression of nucleic acid metabolism. Support for the depression of nucleic acid synthesis at high dosages of Vincristine Sulfate has been reported in animals\(^{13}\) and in tissue cultures.\(^{14}\) Using mouse glioma and L1210 cells, Nesbit\(^{15}\) showed that by using 0.1 mg./Kg. of Vincristine Sulfate there was no effect on DNA or RNA metabolism or depression of oxidative phosphorylation. At higher dosages (7–12 times) a generalized depression of both oxidative phosphorylation and DNA and RNA metabolism was seen with cell death.

Few if any of the normoblastic precursors must have escaped the metaphase arrest. The effect on the granulocytic precursors is minimal. This can best be explained by differences in generation time and labeling indices, and possibly the difference in the ability to escape mitotic arrest without injury.

During the Vincristine Sulfate induced hypoplasia the anticipated block in iron utilization was expressed by the increase in serum iron and almost complete saturation of iron binding. Similar studies after nitrogen mustard,\(^{16}\) and chlorambucil\(^{17}\) induced hypoplasia have been noted. Concomitant with the rebound in marrow erythropoiesis there is a drop in the serum iron as the demand for iron is increased.

**Summary**

The hematopoietic effects of both single and repeated intravenous injections of moderate concentrations of Vincristine Sulfate on rabbit erythropoiesis have been presented. A prompt depopulation of erythroid elements results and is almost completed by 48 hours. Following this depression a rapid rebound of erythropoietic activity occurs. Serum iron values reflect these changes in iron utilization. The reproducibility of these extremes in marrow erythropoietic activity allows one to use the Vincristine Sulfate treated rabbit as an experimental model. It will allow us to study the factors which regulate the balance between erythropoiesis and iron kinetics.

**SUMMARIO IN INTERLINGUA**

Es presentate le effectos hematopoietic de unic e de repetite injectiones intravenose de moderate concentrationes de sulfato de vincristina super le erythropoiese in conilios. Un prompte depopulation de elementos erythroide resulta e es quasi complete in 48 horas. Post iste depression un rapido resalto occurre in le activitate erythropoietic. Le valores seral de ferro reflecte iste alterationes in le utilisation de ferro. Le reproductibilitate de iste extremos in le activitate erythropoietic del medulla rende possibile le uso de conilios tractate con sulfato de vincristina como un modello experimental. Iste modello va render possibile le studio del factores que ha le function de regular le equilibrio inter le erythropoiese e le cinetica de ferro.

**REFERENCES**


Hematopoiesis and Serum Iron Changes Following Vincristine Sulfate

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