The Stathmokinetic Effect of Vincaleukoblastine on Normal Bone Marrow and Leukemic Cells*

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SUMMARY

The effect of Vincaleukoblastine (VLB) on mitosis of normal bone marrow cells (DBA/2 mouse) and leukemic cells (L1210 leukemia) was studied. A single dose of VLB (1 mg/kg) injected intraperitoneally caused a metaphase arrest of cell divisions, in both normal bone marrow and leukemic cells. In the ascitic form of L1210 leukemia a transitory prophase inhibition was also produced.

Vincaleukoblastine (VLB) is a compound recently isolated from *Vinca rosea* Linn. Chemical studies by Gorman *et al.* (6) indicated that it belongs to a new class of dimeric alkaloids containing both indole and dihydriodole moieties.

VLB possesses a marked leukopenic effect, due to depression of leukopoiesis (4, 10). It has been found that this alkaloid is active against several animal tumors and is capable of inducing remission in some human neoplastic diseases (5, 7, 10).

The mechanism of action of VLB is not known. Johnson *et al.* (8) suggested that the compound might act as an antimetabolite by interfering in the metabolic pathways of glutamic acid utilization. Palmer *et al.* (9) have shown that the substance causes a metaphase arrest of cells growing in tissue culture.

The present report deals with the study of the effects of Vincaleukoblastine on the mitotic activity of normal bone marrow and leukemic cells in the mouse.

MATERIALS AND METHODS

Normal DBA/2 mice were used to study the action of VLB on the mitotic activity of normal bone marrow. In preliminary experiments, the dosage of 1 mg of VLB/kg of body weight was selected as the most convenient one for studying the mitotic effect of the drug.

The animals were given injections intraperitoneally of 1 mg of Vincaleukoblastine/kg of body weight and sacrificed in groups of ten animals each, at different time intervals after the administration of the alkaloid. The analysis was carried out on bone marrow preparations made by the Feulgen squash method, following acetic-alcohol fixation. The mitotic indices were determined by counting at least 2000 cells on each slide. The ascitic form of L1210 leukemia, transplanted in DBA/2 mice, was used to study the effect of the compound on the mitotic activity of leukemic cells. All the animals used in this experiment received transplants 5 days before receiving VLB. The transplantation was always made by injecting, intraperitoneally, 0.1 ml. of freshly obtained ascitic fluid containing 1 million cells. The ascitic fluid was obtained from mice which had received a transplantation 5 days before and was diluted with Ringer's solution to the desired concentration of leukemic cells.

VLB was administered intraperitoneally to twelve leukemic animals at a dosage of 1 mg/kg body weight. Six leukemic animals received no treatment and were used as controls. Smears of the peritoneal fluid were made at different time intervals—from 2 hours to 24 hours after the administration of the alkaloid. Smears were stained by Giemsa after methyl alcohol fixation. Each mitotic index was determined by counting at least 2000 leukemic cells.

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RESULTS

Normal bone marrow.—A metaphase arrest of dividing cells was observed in the bone marrow as soon as \( \frac{1}{2} \) hour after the administration of VLB. The number of arrested metaphases increased progressively, following an almost linear pattern from \( \frac{1}{2} \) to 4 hours after the injection of the alkaloid (Chart 1, Fig. 1). No postmetaphase mitotic figures were observed from 1 hour to 4 hours. At the 6th hour, the rate of accumulation of arrested metaphases appeared to be already definitely reduced. At the 8th hour the number of arrested metaphases showed a sharp decline.

L1210 leukemia.—A metaphase arrest of dividing cells was observed in the animals given injections of VLB. The number of arrested metaphases increased from 2 hours to 20 hours after the administration of the alkaloid. No postmetaphase mitotic figures were observed. The accumulation rate of arrested metaphases was low during the first 8 hours and then increased, following an almost linear pattern between 10 and 16 hours after the injection of VLB (Chart 1, Fig. 2). The differential count of mitoses showed that during the first 4 hours, the number of prophases was rather low—in the range of 2.1–2.3 per thousand. It then increased progressively, reaching the level of 7.2 per thousand at 12 hours after the administration of the alkaloid. The percentage of prophases was found to be practically constant from 12 to 20 hours after the treatment. In the control group no significant variations in the mitotic index and the differential count of mitotic figures were observed in seven successive samples of ascitic fluid which were withdrawn during the 32-hour period following the 5th day after transplantation. The mitotic index during this time interval was 37.7 ± 1.36.

Morphology of arrested metaphases.—Various types of arrested metaphases were observed (Figs. 3–9). One type showed the chromosomes to be regularly distributed in the cytoplasm, all converging symmetrically at one focal point (star metaphase) (Fig. 3). In some metaphases of this type, the chromosomes, although distributed around a focal point, did not show a symmetrical arrangement (distorted star metaphases) (Figs. 4–5). Multiple stars were occasionally observed. A second type of arrested metaphase showed the chromosomes scattered irregularly in the cytoplasm (Fig. 6). Some metaphases of this type resembled the so-called “exploded” metaphases. Metaphases, showing toxic changes, were observed particularly 20 and 24 hours after the injection of VLB. In these, various degrees of chromosome clumping could be observed (Figs. 7–9).

Some metaphases showed all chromosomes clumped in a pyknotic mass, and they resembled the so-called “ball” metaphases.

DISCUSSION

The results obtained in normal bone marrow demonstrated that VLB possesses the property of arresting cell division at the metaphase stage (stathmokinetic effect). The stathmokinetic effect appeared very shortly after the administration of the alkaloid and continued for a few hours, as indicated by the progressive increase in arrested metaphases, from \( \frac{1}{2} \) to 4 hours. The accumulation rate of arrested metaphases fell rapidly at the 8th hour.

The pattern of bone marrow metaphase accumulation after the administration of VLB is definitely similar to the one observed after colchicine treatment. In previous studies, we have been able to demonstrate that the pattern of bone marrow metaphase accumulation after colchicine treatment correlates well with the concentration of this alkaloid in the bone marrow (1). It is also possible that, in the animals treated with VLB, the marked fall in metaphase accumulation at the 8th hour after treatment might depend on the metabolism of the substance in the bone marrow.

In the L1210 leukemia treated with VLB, two main effects were observed: (a) a metaphasic arrest
of dividing cells and (b) a prophase effect, as indicated by the changes in the prophase percentage at different times after the injection of the alkaloid.

The number of prophase was very low during the first 4 hours after the injection of the alkaloid. The low rate of accumulation of metaphases during the first 8 hours was probably due to the reduced number of cells entering mitosis. From 10 hours to 20 hours no prophase inhibition was observed, and the number of arrested metaphases increased almost linearly from 10 hours to 16 hours after the treatment. Some of the arrested metaphases undergo degeneration, and many degenerating cells were observed in smears of the peritoneal fluid made 24 hours after the injection of VLB (Fig. 10). The mitotic changes observed in the ascitic form of L1210 leukemia after treatment with VLB are similar to the changes observed after colchicine treatment (2).

When this study was completed, a paper by J. H. Cutts (3) was published on the effect of VLB on mitosis in vivo. Our findings are in good agreement with the results published by Cutts in regard to the stathmokinetic effect of VLB in both normal and leukemic cells in vivo. However, in contrast with his observations, it is our finding that, in the ascitic form of L1210 leukemia, VLB injected intraperitoneally caused transitory inhibition of prophase during the first 6–8 hours. Other observations by Cutts, concerning the effect of VLB on normal bone marrow, are in further contrast with our results. The number of arrested metaphases in the bone marrow of the normal rat increased, according to Cutts, from 2 hours until 12 hours after injection. Inspection of his data revealed that, between the 4th and 6th hour, the rate of accumulation of metaphases showed a minor decline which, however, was markedly reversed between the 6th and 12th hour, with a maximum number of arrested metaphases at 12 hours. We found that, although we could demonstrate a similar decrease in the rate of metaphase accumulation between 4 and 6 hours, it was not transient, nor was it reversed; and our data differ markedly from those of Cutts in that, in our studies in DBA/2 mice, the maximum number of arrested metaphases was achieved at 6 hours after treatment, with a rapid decline thereafter. To obtain a better evaluation of the difference between our mouse data and those of Cutts in the rats, additional observations on twelve mice, sacrificed 12 hours after the injection of VLB, were carried out. These observations revealed that, at the 12th hour after the administration of the alkaloid, the number of arrested metaphases in the bone marrow was 27.8 per thousand, while at the 6th hour it was 85.0 per thousand, thus confirming our findings and revealing, once again, a sharp drop in the rate of metaphase accumulation after 6 hours, indicating that after this time there may no longer be a stathmokinetic effect by VLB in the mouse bone marrow.

In conclusion, the results of this investigation showed that Vincaalkoblastine belongs to the metaphase poisons of mitosis, but also that it is able, under certain conditions, to cause prophase inhibition.

The effect of Vincaalkoblastine on the mitotic activity of both normal bone marrow cells and leukemic cells in vivo is similar to the effect of colchicine.

REFERENCES

FIG. 1.—Arrested metaphases in the bone marrow of a DBA/2 mouse, 4 hours after the injection of VLB. Squash preparation, Feulgen. X460.

FIG. 2.—Arrested metaphases in the ascitic fluid of a DBA/2 mouse with L1210 leukemia, 16 hours after the injection of VLB. Smear, Giemsa. X800.

FIG. 3.—L1210 leukemia 12 hours after the injection of VLB. Arrested metaphase (star metaphase). Smear, Giemsa. X2200.

Figs. 4, 5.—L1210 leukemia 12 hours after the injection of VLB. Arrested metaphases resembling the so-called distorted star metaphase. Smear, Giemsa. X2200.

FIG. 6.—L1210 leukemia 12 hours after the injection of VLB. Arrested metaphase showing chromosomes irregularly scattered in the cytoplasm. Smear, Giemsa. X2200.

Figs. 7–9.—L1210 leukemia 20 hours after the injection of VLB. Arrested metaphases showing various degrees of chromosome clumping. Smear, Giemsa. X2200.

FIG. 10.—L1210 leukemia 24 hours after the injection of VLB. Cells showing marked degenerative changes. Smear, Giemsa. X2200.
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