Studies on the Antimitotic Activity of Leurocristine (Vincristine)

By GIUSEPPE CARDINALI, GIULIANA CARDINALI AND MOSTAFA ABOUL ENEIN

GREAT INTEREST has recently been aroused in a group of alkaloids, extracted from *Vinca rosea* Linn, because of their antimitotic and antitumor activity. Four principal alkaloids have been isolated from *Vinca rosea*: vincaleukoblastine (vinblastine), leurosine, leurosidine, and leurocristine (vincristine). Vinblastine has been more extensively studied, both in vitro and in vivo. It has been demonstrated that it is an antimitotic agent of the colchicine type (stathmokinetic agent) and that it is active against some animal tumors. Vinblastine has also shown some favorable effects in the treatment of certain forms of neoplastic diseases in man, particularly in the treatment of Hodgkin's disease.

More recently, vincristine has also been found to possess antitumor activity, both in animals and in humans. Encouraging results have been reported in the treatment of lymphoma and acute lymphatic leukemia. The aim of this investigation was to study the effect of vincristine on cell proliferation in normal bone marrow and leukemic cells.

**Materials and Method**

DBA/2 mice, of both sexes, weighing 20-24 Gm., were used in all experiments.

**Preliminary experiment.** Four groups of four mice each were injected intraperitoneally with 0.05 mg./Kg., 0.08 mg./Kg., 0.25 mg./Kg. and 0.50 mg./Kg. of vincristine, respectively. The animals were sacrificed 4 hours after the administration of the alkaloid. The marrow of both femurs was collected and fixed in 70 per cent methyl alcohol. Bone marrow preparations were made by the Feulgen-squash method. The details of the technic were described in a previous paper. Mitotic indices were determined by counting at least 2000 cells on each slide.

**Experiment 1.** Six groups of 10 DBA/2 mice each were injected intraperitoneally with 0.5 mg./Kg. of vincristine. Each group of animals was sacrificed at different time intervals after the treatment. A group of 10 DBA/2 mice received no treatment and was used as a control group.

Bone marrow preparations were made by the Feulgen-squash method. Mitotic indices were determined by counting at least 2000 cells on each preparation.

**Experiment 2.** Two groups of 12 and 6 DBA/2 mice, respectively, were transplanted with the ascitic form of L1210 leukemia. The number of cells used for transplantation was 1 x 10⁶. Five days after transplantation, the first group of 12 animals was injected intra-
DOSE - mg/Kg

0.08 0.25

DOSE - mg/Kg

0 0.05 0.08 0.25 0.5

Fig. 1.—Bone marrow mitotic indices in DBA 2 mice 4 hours after injection of different doses of vincristine.

peritoneally with 0.5 mg./Kg. of vincristine. The second group of animals received no treatment and was used as a control group. Peritoneal fluid was withdrawn at different time intervals after treatment, smeared as usual, and stained by Giemsa. Mitotic indices were determined by counting at least 2000 cells on each slide.

RESULTS

Preliminary experiment. In the preliminary experiment, the presence of arrested metaphases was observed in all bone marrow preparations. The number of arrested metaphases appeared to be related to the dosage employed (fig. 1). The highest values of mitotic index were found in the animals treated with 0.5 mg./Kg. of vincristine. In these animals no postmetaphase figures were observed, while they were present in the animals treated with 0.05 mg./Kg. and 0.08 mg./Kg. and, occasionally, also in the animals treated with 0.25 mg./Kg. of vincristine.

Experiment 1. Arrested metaphases were observed in the bone marrow of the treated animals as soon as one-half hour after the administration of 0.5 mg./Kg. of vincristine. The number of arrested metaphases increased from one-half hour to 6 hours after the treatment, following an almost linear pattern from one-half hour to 4 hours (figs. 2 and 3). No postmetaphase figures were generally observed in the time interval from 1 hour to 4 hours after the injection of vincristine. At the eighth hour after the administration of the alkaloid, the mitotic index showed a marked fall.

Experiment 2. The leukemic animals were followed from 1 hour until 16 hours after the administration of the alkaloid. Arrested metaphases were observed in the leukemic cells as soon as 1 hour after the treatment. The mitotic index increased progressively from the first hour to the 16th hour. The rate of accumulation of arrested metaphases was relatively low during the first
Fig. 2.—Bone marrow mitotic indices in DBA 2 mice at different time intervals after injection of vincristine (0.5 mg., Kg.).

Fig. 3.—Arrested metaphases in the bone marrow of a DBA/2 mouse 4 hours after the injection of vincristine (0.5 mg. Kg.). Squash preparation, Feulgen.
4 hours and then increased following an almost linear pattern between 8 and 12 hours, (figs. 4 and 5). Postmetaphase figures were not observed in the period of time between 1 hour and 12 hours, while they were occasionally found in the preparations made 16 hours after the administration of vincristine.

The number of prophases was relatively low (3.5 per 1000) at the first hour, and then progressively increased, reaching the level of 8.1 per 1000 at the eighth hour. The number of prophases remained practically constant from the eighth hour until the 16 hour after treatment. In the control group, the mitotic index remained at an almost constant level throughout the time interval of the experiment.

DISCUSSION

The results obtained showed that vincristine is, like colchicine and vinblastine, a stathmokinetic agent. Doses at least twice as large are required to obtain a comparable degree of metaphase arrest with colchicine, desacetyl-methylcolchicine (demecolcine) and vincaleukoblastine (vinblastine).32-34 The stathmokinetic effect of vincristine on bone marrow cells was very similar to the one observed after colchicine, demecolcine, and vinblastine. The similarity of the pattern of bone marrow metaphase accumulation in the animals treated with Vinca rosea Linn alkaloids and in the animals treated with colchicine suggests that all these alkaloids accumulate rapidly in the bone marrow and are also rapidly eliminated from this tissue. In a previous study we have observed that the level of colchicine concentration increases
In the leukemic cells, a complete metaphase arrest was observed as soon as 1 hour after the injection of 0.5 mg./Kg. of vincristine. The rate of accumulation of arrested metaphases was relatively low during the first 4 hours. This was very likely due to the transitory prophase inhibition. We have observed a similar phenomenon also in the L1210 leukemia (ascitic form) treated with colchicine or vinblastine. This probably depends on the fact that soon after the injection of these alkaloids in the peritoneal cavity, their local concentration is relatively high, thus causing a depression of mitotic activity. This is not surprising because it is well known that colchicine at high dose level may cause prophase inhibition.

The morphology of arrested metaphases in the animals treated with vincristine was also very similar to that described in the animals treated with vinblastine. In some arrested metaphases the chromosomes appeared arranged around a focal point (star metaphases), while in others the chromosomes were irregularly scattered in the cytoplasm. In some cells, the chromosomes showed a varying degree of clumping. Three-group and four-group metaphases were observed perhaps more frequently than in the animals treated with colchicine or vinblastine (fig. 6).
Fig. 6.—Different morphologic aspects of arrested metaphases in L1210 leukemia treated with vincristine (0.5 mg./Kg.). Smear, Giemsa.
Our findings on the stathmokinetic effect of vincristine in vivo are in good agreement with the results recently obtained in vitro by Palmer and Warren.31 It is interesting to note that while demecolcine, vinblastine, and vincristine possess the same type of antimitotic activity, they seem to differ quite markedly in their antitumor effect. For instance, demecolcine is known to possess some activity against chronic myeloid leukemia, while vincristine seems to be active against acute lymphatic leukemia.27–30 Vinblastine, on the other hand, seems to be of little use as an antileukemic agent with, perhaps, some potential activity against monocytic leukemia.13,22 One can wonder what the relationship is between the antimitotic and the antitumor activity in this group of substances. In the case of vinblastine it has been claimed that it might act also as an antimetabolite,12 and a reversal of the cell inhibitory activity of vinblastine by glutamic acid or tryptophan has been observed by Johnson et al.12 and by Cutts.7 We were not able to obtain any significant reversal of the antimitotic effect of vinblastine in both normal and leukemic cells by the use of these two amino acids.35 At present, the question of the relationship between antitumor and antimitotic effect of these alkaloids has no definite answer. It is possible that the antitumor effect is completely independent from the antimitotic effect, but it is equally possible that the two phenomena are more or less correlated.

**Summary**

The effect of leurocristine (vincristine) on cell division was studied in normal bone marrow and leukemic cells (L1210 leukemia) of DBA/2 mice. It was found that vincristine possesses the ability to arrest mitosis at the metaphase stage. The accumulation of arrested metaphases in the normal bone marrow and in leukemic cells of the animals treated with vincristine followed a pattern similar to the one observed after treatment with colchicine or vincaleukoblastine (vinblastine).

**Summario in Interlingua**

Le effecto de leurocristina (vincristina) super le division cellular esseva studiate in cellulas de medulla ossee normal e leucemic (leucemia L1210) de muses DBA/2. Esseva constatate que vincristina possede le capacitate de arrestar le processo mitotic in le stadio del metaphase. Le accumulation de arrestate metaphases in le medulla ossee normal e in cellulas leucemic de animales tractate con vincristina sequeva un ordine simile a illo observate post le tractamento con colchicina o vincaleu koblastina (vinblastina).

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