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The Catharanthus Roseus (Vinca Rosea) Alkaloids: A New Class of Stathmokinetic Agents

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Interest in stathmokinetic agents is increased in the last few years because of the discovery of a new group of stathmokinetic compounds, which have shown to possess a definite antitumor activity. This new group is formed by a number of alkaloids of which the best known are *vinblastine* (VLB) and *vincristine* (VCR). The chemical structure of these 2 alkaloids has been elucidated by Neuss *et al.* (1962). VLB and VCR differ in the radical R attached to the vindoline moiety which is a *methyl* group in Vinblastine and a *formyl* group in Vincristine (Fig. 1).

These 2 alkaloids are currently referred to as *Vinca rosea* alkaloids, but actually they are extracted from a pantropical plant which does not belong to the genus *Vinca* but to the genus *Catharanthus* (Pichon, 1948; Lawrence, 1959; Fernsworth, 1961). The correct botanical name for this plant is *Catharanthus roseus* (L.) G. Don and not Vinca rosea (Fig. 2). The differences between the two genera Vinca and Catharanthus are summarized in Tab. 1. Unfortunately, to the great disappointment of botanists, the denomination of Vinca rosea alkaloids continues to be used by medical people for VLB, VCR and related compounds.

In 1961 indipendently from the work of other authors we were able to demonstrate that VLB is a stathmokinetic agent in vivo, and in 1962 we demonstrated that also VCR is a metaphase blocking agent in vivo (Cardinali *et al.*, 1961, 1963).

The antimitotic effect of these alkaloids was studied both on normal bone marrow and tumor cells (Fig. 3 and 4).

The accumulation of arrested mitoses, in the bone marrow of animals injected with VLB, is a linear function of time during the first 4-6 hours after the administration of the compound. Arrested metaphases can be noticed in the bone marrow of the treated animals as soon as ½ an hour following the injection of VLB (Cardinali *et al.*, 1961). A similar pattern is observed in the animals treated with VCR (Cardinali *et al.*, 1963).

The stathmokinetic effect is dose dependent up to a certain level (Fig. 5). With

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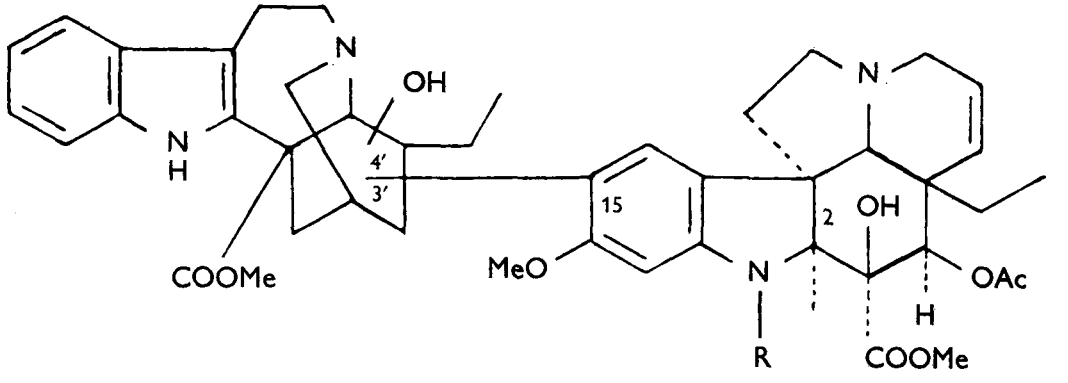

Vinblastine I, R = CH_3

III, R = CHO **Vincristine**

Fig. 1

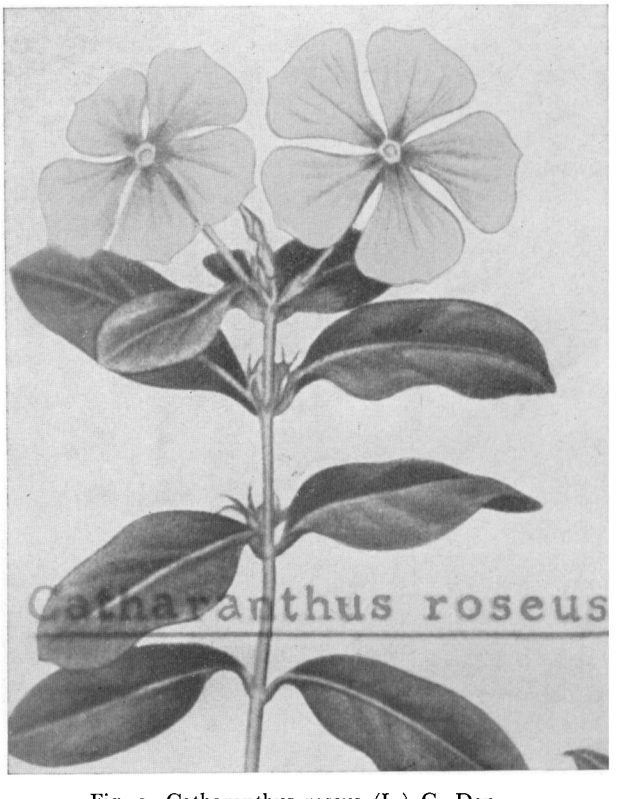

Fig. 2. Catharanthus roseus (L.) G. Don

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Tab. 1. Technical characters of the floral structures of Vinca and Catharanthus

	Vinca	Catharanthus
Type of plant	Woody subshrub, the stems persisting many years	Annual or perennial herb, the stems dying to ground or short-lived
Foliage	Persistent, the plant ever green, the leaves often leathery	Deciduous, not persisting when the plant is perennial, the leaves softherbaceous
Flowers	Solitary in leaf axils	Usually 2-3 in cymose axillary clusters
Corolla	Infundibular, tube funnel form; mouth open, or closed by scale-like appendages	Salverform, the tube narrowly cylin drical; the mouth closed by bristlelike hairs
Ovaries	Provided with nectaries much shorter than ovary	Provided with nectaries as long as or longer than the ovary
Stigma	Lacking any thin hyaline basal "petti-coat"	Thin hyaline basal "petticoat" present
Style	Broadest at apex, immediately below the stigma	Uniformly slender, not becoming broad-er towards apex
Follicle	Mostly 6-8-seeded	Mostly 15-30 (or more)-seeded

(After Lawrence, 1959)

VCR the maximum stathmokinetic effect in the mice was obtained with the dose of 0.5 mg/kg (Cardinali *et al.*, 1963).

Comparative studies conducted with Colchicine, VLB and VCR in the mouse and the rat showed that the three alkaloids have a similar effect on bone marrow cells (Cardinali and Cardinali, 1967).

The pattern of metaphase accumulation in the mouse bone marrow is similar for the three alkaloids. The mitotic index is a linear function of time during the first 4-6 hours. The maximum value is reached at the sixt hour, and then the mitotic index declines quite sharply (Fig. 6).

In the rat, the mitotic index is a linear function of the time during the six hours after the administration of the alkaloids (Fig. 7). The animals treated with VCR show a greater accumulation of arrested metaphases, in comparison with the animals treated with colchicine or VLB. The rather rapid decline of the stathmokinetic effect in the bone marrow is, very likely, due to the physiological disposition of these alkaloids.

In studies conducted in the WR rats treated with a single injection of colchicine we have been able to demonstrate that the concentration of this alkaloid in the bone marrow increases from 1st to the 4th hour after its administration and than falls to a low level at the sixth your. No colchicine is detectable in the bone marrow of ani-

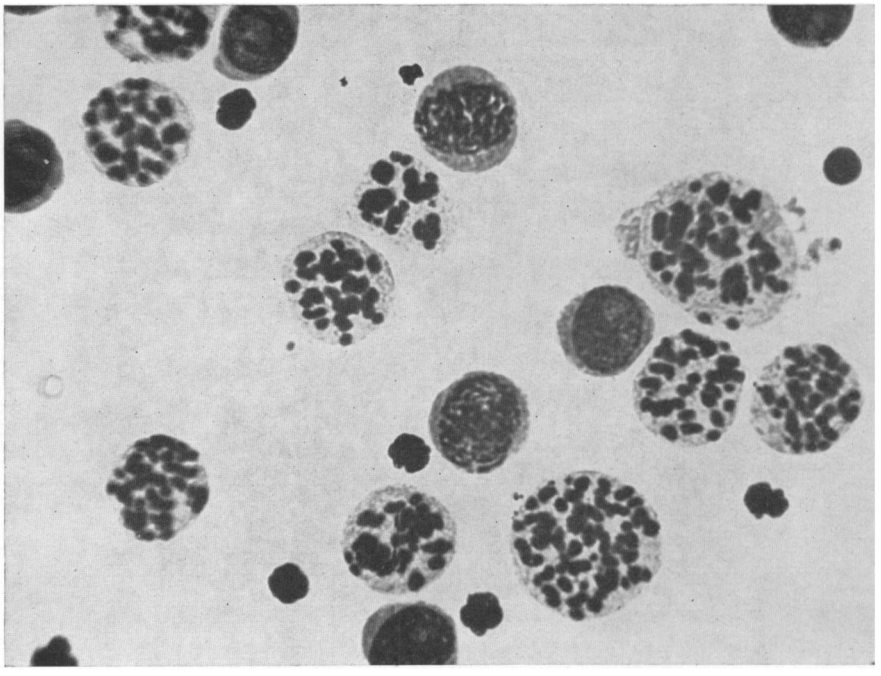

Fig. 3. Ehrlich ascites carcinoma. Arrested metaphases 16 hours after the injection of vinblastine (1 mg/kg)

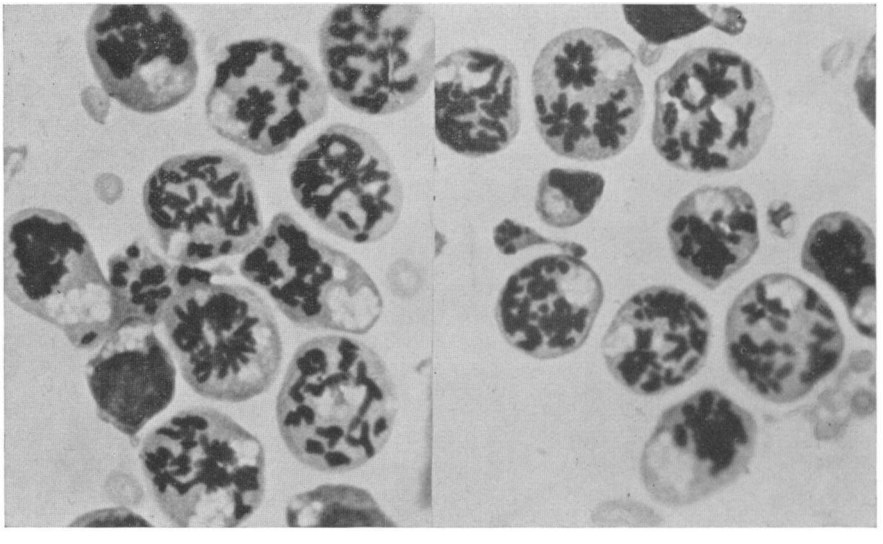

Fig. 4. L1210 leukemia (ascitic form). Arrested metaphases 12 hours after the injection of vincristine (0.5 mg/kg)

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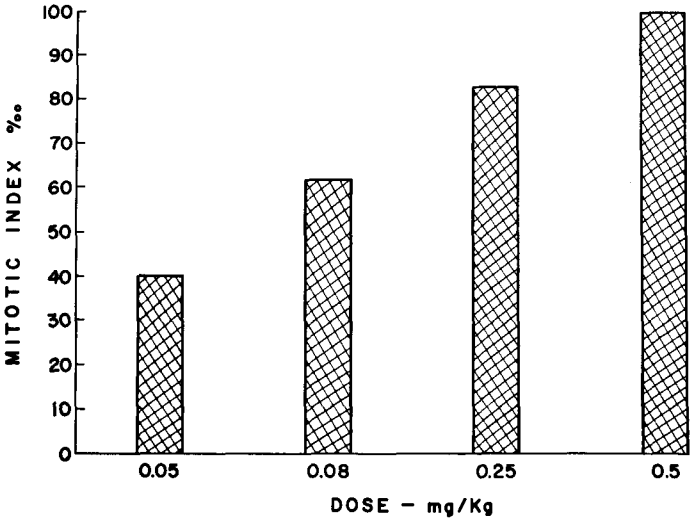

Fig. 5. Bone marrow mitotic indices in DBA/2 mice 4 hours after injection of vincristine (Courtesy of Grune and Stratton. *From* G. Cardinali *et al.* Blood, **21:** 102, 1963)

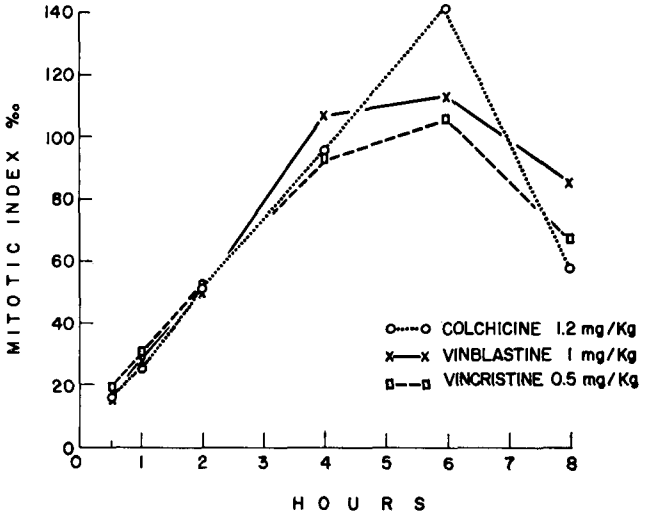

Fig. 6. Mouse bone marrow mitotic indices after colchicine, vinblastine, or vincristine (Courtesy of Karger. *From* G. Cardinali and G. Cardinali. Proc. 10th Congr. Europ. Soc. Haemat. Part II, p. 831, 1967)

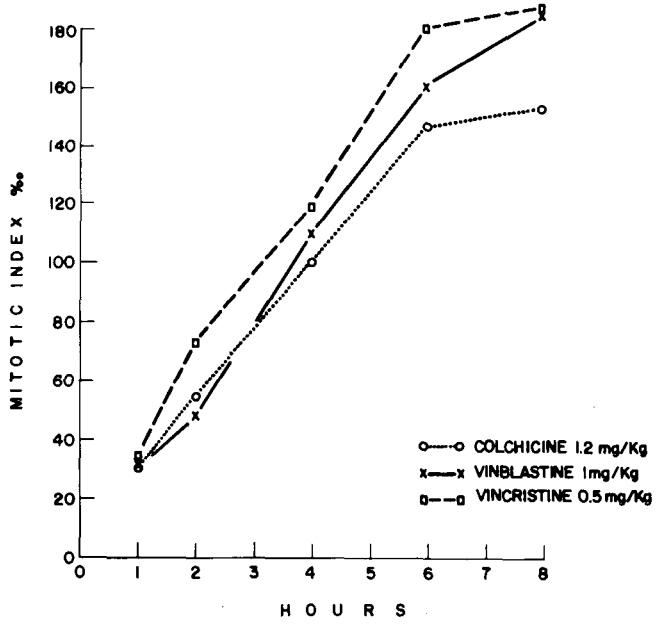

Fig. 7. Rat bone marrow mitotic indices after colchicine, vinblastine, or vincristine (Courtesy of Karger. From G. Cardinali and G. Cardinali. Proc. 10th Congr. Europ. Soc. Haemat. Part II, p. 831, 1967)

mals sacrified 8 hours after the injection of the drug. At any rate, the pattern of accumulation of colchicine in the bone marrow is similar to the pattern of metaphase accumulation (Cardinali *et al.*, 1961).

It must be pointed out that at high concentrations, stathmokinetic agents may inhibit cells to enter mitosis. In fact in L1210 leukemia (ascitic form) treated with VLB or VCR the accumulation of arrested mitoses is rather low during the first 4 hours after the injection of the alkaloids (Cardinali *et al.*, 1961, 1963). The same phenomenon is observed in the leukemic animals treated with colchicine (Fig. 8). During the first 4-6 hours after the treatment the number of prophases is very low. In all these experiments the stathmokinetic agent was injected in the peritoneal cavity of the animals carrying the ascitic form of L1210 leukemia. Therefore, during the first few hours after the introduction of the agent, the proliferating cells were exposed to a high concentration of the drug, thus causing a prophasic inhibition.

Of special interest to us were the findings in the Syrian hamster treated with VLB and VCR (Cardinali *et al.*, 1961, 1967; Mehrotra and Cardinali, 1965). This animal was used because it was known that it is resistant to the action of colchicine.

Comparative studies conducted with colchicine, VLB and VCR in the bone marrow of the hamster, showed that colchicine, at the dose of 1.2 mg/kg, is ineffective

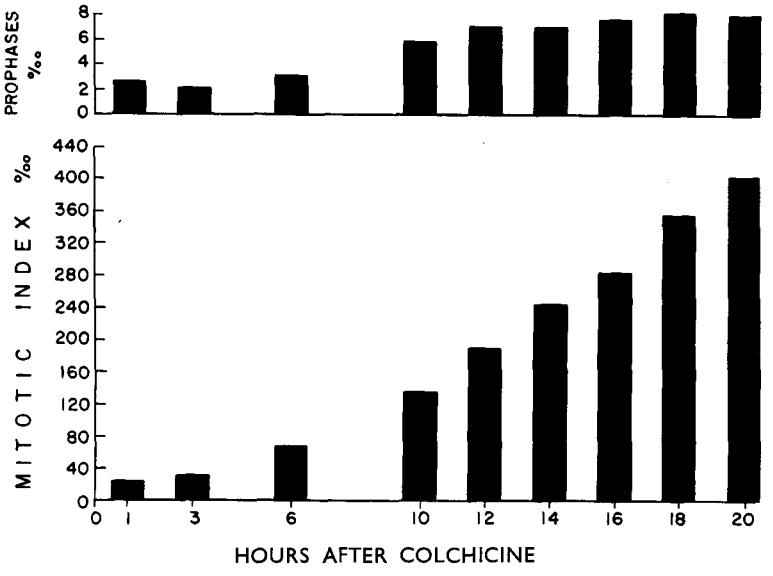

Fig. 8. Mitotic indices in L1210 leukemia after treatment with colchicine (1.2 mg/kg)

in Syrian hamster. At the dose level of 2.4 mg/kg, only part of the hamster bone marrow dividing cells were arrested at the metaphase stage. No greater stathmokinetic effect was obtained by increasing the dosage of colchicine up to 7.2 mg/kg. On the other hand, a complete mitotic block was observed in the animals treated

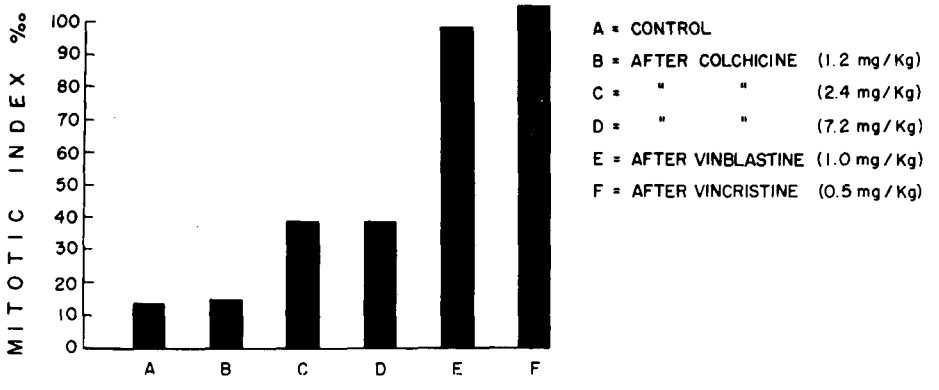

Fig. 9. Bone marrow mitotic indices in syrian hamster before and after treatment with colchicine, vinblastine, or vincristine
(Courtesy of Karger. From G. Cardinali and G. Cardinali, Proc. 10th Congr. Europ. Soc. Haemat. Part II, p. 381, 1967)

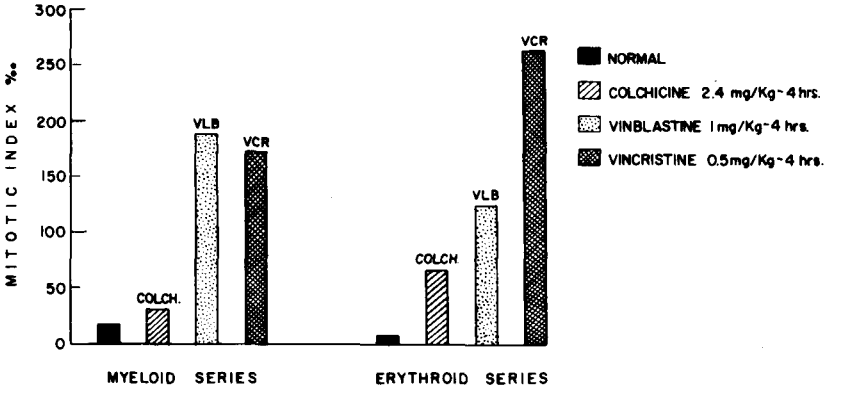

Fig. 10. Bone marrow mitotic indices in normal syrian hamsters and hamsters treated with single doses of colchicine, vinblastine, or vincristine
(Courtesy of Karger. *From* G. Cardinali and G. Cardinali. Proc. 10th Congr. Europ. Soc. Haematol., part II, p. 831, 1967)

with VLB and VCR (Fig. 9). Differential mitotic counts on the myeloid and erythroid series showed (Fig. 10) that:

1) in the animals treated with colchicine the partial mitotic arrest was mainly due to a block of erythroblasts (Fig. 11);

2) in the animals treated with VLB practically all bone marrow dividing cells were arrested. The total number of accumulated metaphases was higher in the white series than in the red series (Fig. 12). Also in the hamster treated with VCR almost all dividing cells were arrested, but, while the number of arrested metaphases in the myeloid series was almost the same as in the animals treated with VLB, the number of accumulated metaphases in the erythroid series was much higher than in the hamsters treated with VLB (Fig. 13).

The high mitotic index in erythroblasts, observed in the hamster treated with VCR, is difficult to explain, and the effect of this alkaloid on the erythroid series needs to be further explored.

As in vivo studies, also in vitro studies showed that colchicine, demecolcine, VLB and VCR possess the same type of antimitotic activity. However, these alkaloids seem to differ quite markedly in their antitumor acticity. For instance, democolcine is known to have some activity against chronic myeloid leukemia; VLB, which is a good drug for treatment of Hodgkin disease, is considered to be of little use in leukemia, while VCR is active against acute leukemia. One then wonders what the relationship is between the antitumor and the antimitotic activity in this group of substances. It has been suggested that Catharanthus alkaloids might act as antimetabolites and it has been reported that the effect of VLB may be reversed by some aminoacids, in particular glutamic acid and tryptophan (Cutts, 1961; Jonhson *et al.*, 1963).

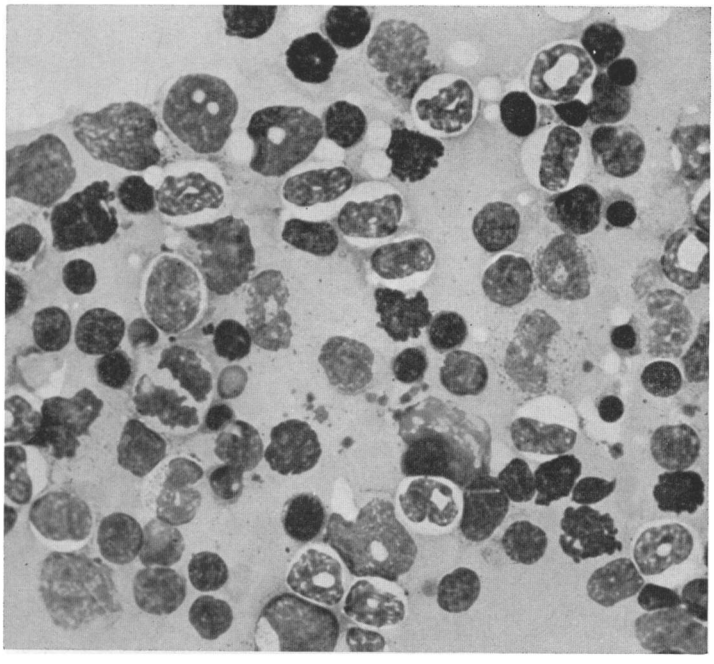

Fig. 11. Bone marrow smear obtained from a Syrian hamster injected with colchicine (2.4 mg/kg). Note the presence of some arrested metaphases and of one post-metaphase figure in a myelocyte

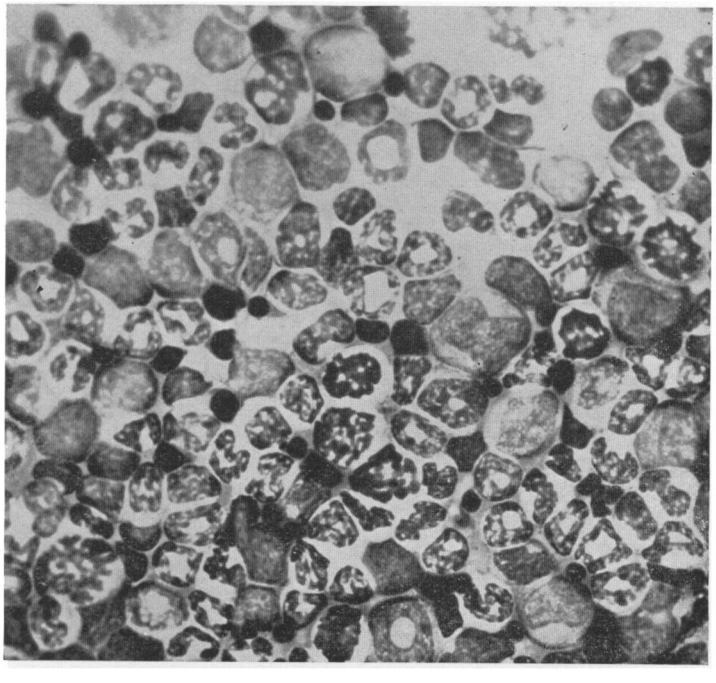

Fig. 12. Bone marrow smear obtained from a Syrian hamster injected with vinblastine (1 mg/kg). Arrested metaphases are prevalent in the myeloid series

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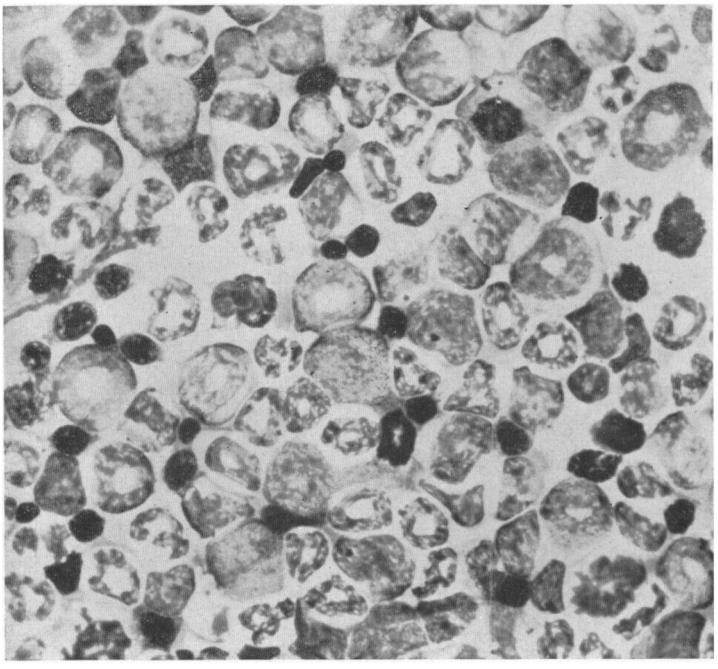

Fig. 13. Bone marrow smear obtained from a Syrian hamster injected with vincristine (0.5 mg/kg). Arrested metaphases are prevalent in the erythroid series

We have studied the effect of these aminoacids on the antimitotic effect of VLB on L1210 leukemia (Cardinali *et al.*, 1963). The aminoacids were given in 2 doses of 100 mg/kg each; the first dose was given 1 hour before and the second dose one hour after VLB. No significant differences were found between the mitotic indices in the animals treated with VLB alone and those treated with VLB plus glutamic acid or tryptophan (Cardinali *et al.*, 1963). Our findings are in contrast with those of Cutts who reported that glutamic acid and tryptophan can modify the stathmokinetic effect of VLB.

However other authors have shown that these 2 aminoacids may reverse some effects of VLB and VCR. Creasey (1964) has shown that glutamic acid may, at least partially, reverse the inhibitory effect of Catharanthus alkaloids on RNA synthesis. Johnson has demonstrated that glutamic acid may reverse the antileukemic and the toxic effects of VLB (1963).

Vaitkevicius (1962) and also Armstrong (1962) reported that the leukopenic effect of VLB may be reversed by aspartic acid. It seems, however, that large doses are needed to get this protective effect and these high doses are toxic by themselves so that the use of this aminoacid is unpractical.

Other aminoacids have been claimed to be active in vitro in reversing the effect of VLB, like aspartic acid, o-chetoglutaric acid, ornitine, citrulline, arginine. It seems, however, that also with glutamic acid only a partial reversion may be obtained

in particular experimental conditions. It has been advanced the hypotesis that the metaphasic arrest caused by VLB and VCR is only a secondary phenomenon and that these alkaloids act mainly as antimetabolites. We do not think that the stathmokinetic effect of these drugs may be dimissed so simply. It is possible that the anti-tumor effect of VLB and VCR is not entirely dependent on their antimitotic activity, but it is likely that such activity plays a role in the effects of VLB and VCR on tumor growth and bone marrow activity. The observation of Marmont and Damasio (1965) that there is a correlation between the degree of metaphasic arrest and the clinical response in patients treated with VCR is in keeping with this hypotesis.

Summary

Vinblastine (VLB) and vincristine (VCR) are two alkaloids (extracted from the plant Catahranthus roseus) which possess a definite antitumor activity. The antitumor activity. The antimitotic action of these two alkaloids was studied both on normal bone marrow and leukemic cells. Comparative studies showed that they are, like colchicine, spindle poisons. They, however, differ from colchicine and its derivatives in some of their biological activities.

The problem of the relationship between antitumor and antimitotic activity of VLB and VCR is discussed.

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RIASSUNTO

La vinblastina (VLB) e la vincristina (VCR) sono due alcaloidi, estratti dal Catharanthus roseus, i quali possiedono una notevole attività antitumorale. L'effetto di questi due composti sulla proliferazione cellulare è stato studiato su cellule midollari normali e su cellule leucemiche. Ricerche comparative hanno dimostrato che la VLB e la VCR sono, come la colchicina, agenti statmocinetici. I due alcaloidi però differiscono dai composti colchicinici in alcune delle loro attività biologiche. I rapporti tra attività antimitotica e attività anti-tumorale della VLB e della VCR vengono discussi.

RÉSUMÉ

La vinblastine (VLB) et la vincristine (VCR) sont deux alcaloïdes, extraits du Catharanthus roseus, qui possèdent une remarquable activité antitumorale. L'effet de ces deux composés sur la prolifération cellulaire a été étudié sur des cellules de la moelle osseuse, normales et léucémiques. Des études de comparation ont démontré que la VLB et la VCR sont des agents stathmokinétiques, comme la colchicine. Les deux alcaloïdes, toutefois, diffèrent des composés colchiciniques, en ce qui concerne quelques unes de leurs activités biologiques. Les rapports entre activité antimitotique et activité antitumorale de la VLB e de la VCR sont discutés.

ZUSAMMENFASSUNG

Vinblastina (VBL) und Vincristina (VCR) sind zwei aus dem Catharanthus roseus gewonnene Alkaloide, die erhebliche antitumorale Wirkung besitzen. Der Effekt dieser beiden Präparate auf die Zellproliferation wurde bei normalen und leukämischen Knochenmarkszellen untersucht. Vergleichende Forschungen ergaben, dass VLB und VCR, wie Colchicina, statmokinetische Wirkstoffe sind.

Die beiden Alkaloide unterscheiden sich jedoch von den Colchicinverbindungen in einigen ihrer biologischen Wirkungen. Es wird das Verhältnis zwischen der antimitotischen und der antitumoralen Wirkung von VLB und VCR erörtert.