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Correlation of the Clinical Neurotoxicity of the Vinca Alkaloids Vincristine, Vinblastine, and Vindesine with their Effects on Cultured Rat Midbrain Cells

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Summary. *Clinical experience with three vinca alkaloids currently in use as antineoplastic agents has shown a difference in the degree of peripheral neurotoxicity manifested by these compunds: vincristine > vindesine > vinblastine. This phenomenon may reflect differences in pharmacokinetics and/or the differential response of the nerve tissue itself. Differences in pharmacokinetics can be avoided by studying the direct effects of the vinca alkaloids on primary cultures of neuronal and glial cells. Vincristine at a dose as low as 0.004 μg/ml affects the cells with processes in cultures of dissociated newborn rat midbrain. In 3-day-old cultures, after 24 h of drug treatment there is a loss of processes and swelling of the cell body. We have used this observation as the basis for a quantitative assay of the toxicity of a series of vinca compounds, and have found that for a dose range of 0.1 – 0.004 μg/ml the relative toxicity of vincristine, vinblastine, and vindesine in this system correlates with their relative clinical neurotoxicity. Validation of the predictive elements of this system awaits clinical experience with novel vinca compounds.*

Introduction

Peripheral neurotoxicity, manifested as suppression of Achilles-tendon reflex, paresthesia in the extremities, and gastrointestinal distress, presents a serious limitation to the use of the vinca alkaloid vincristine as a chemotherapeutic agent in the treatment of some forms of cancer. There is no known therapy for this peripheral nerve dysfunction other than reduction of the dose or discontinuation of the medication [15]. Of the three vinca alkaloids currently used in clinical treatment of neoplasms, vincristine is the most neurotoxic. Neurotoxicity is a less serious problem with vinblastine for which marrow suppression is the dose-limiting factor [15]. Vindesine, a new vinca compound [1] undergoing clinical trial, appears to be moderately neurotoxic [8]. A system suitable for study of the factors underlying clinical differences and predictive of neurotoxicity would augment the development of novel vinca molecules by permitting early exclusion of those derivatives showing neurotoxic potential. Neurotoxicity is, however, difficult and time-consuming to assess in animal models, especially rodents, in which vincristine produces a severe myopathy instead of neuropathy [15].

We have observed that vincristine, at a dose as low as 0.004 μg/ml, affects cells with processes in dissociated cultures of newborn rat midbrain. In 3-day-old cultures, after 24 h of exposure to vincristine there is a loss of processes and swelling of the cell body. We have used this observation as the basis for a quantitative assay of the toxicity of a series of vinca compounds, the results of which correlate with the relative clinical neurotoxicity of vincristine, vinblastine, and vindesine.

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Methods

Midbrain Culture

The dorsal area of the brains from 2- to 5-day-old Wistar rats (sacrificed with methoxyflurane anesthesia followed by exposure to CO_2) was exposed, and cuts were made to the ventral surface immediately posterior and anterior to the corpora quadrigemina. This tissue area (referred to as midbrain) was removed aseptically, cleaned of membranes and blood as much as possible, finely minced, and incubated with slow stirring in Seto and Rounds' Simple Trypsin Solution [11] at 4° C for 30 min. The digested tissue was aspirated ten times to break up tissue chunks, and the larger pieces were allowed to settle out by gravity. The supernatant was removed and centrifuged. The pelleted cells were washed once in complete growth medium consisting of Ham's Nutrient Mixture F_{12} (Grand Island Biological Company) supplemented with 20% fetal calf serum (Grand Island Biological Company). The cells were planted in the wells of Linbro Disposo Trays (Linbro Chemical Company, Inc.), with 1 ml per well and ten midbrains per tray of 24 wells.

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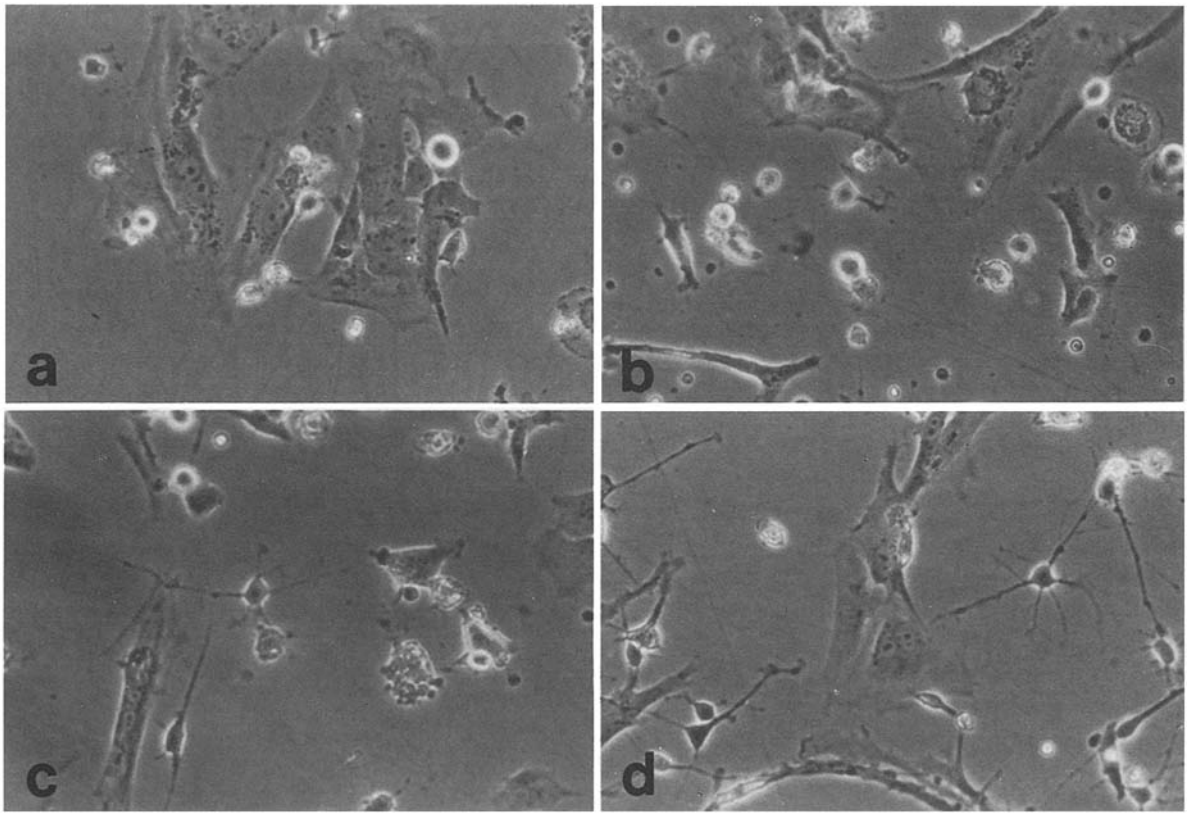

Fig. 1a—d. Cultured, dissociated, newborn rat midbrain cells 24 h after treatment at a dose of 0.02 μg/ml on day 3 with vincristine **a**, vindesine **b**, or vinblastine **c**. Control cells are also shown **d**. Phase contrast magnification × 216

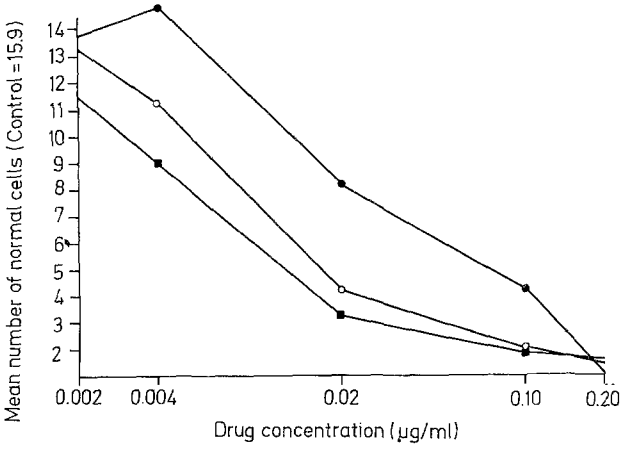

Fig. 2. Toxic response of cultured rat midbrain cells to the vinca alkaloids vincristine (■), vindesine (○), and vinblastine (●)

Toxicity Assay

Vincristine, vinblastine, and vindesine (Eli Lilly and Company) were dissolved in F_{12} at 100 X concentration and added to the cells on the third day of culture in a volume of 10 μl per well. After 24 h three microscope fields per well were photographed. The numbers of small, phase-dark, ovoid cells with processes per field were counted and compared with the numbers in nontreated controls.

Results

A typical 4-day-old culture of dissociated newborn rat midbrain is shown in Fig. 1d. These cultures contain several cell types, including phase-bright, vacuole-laden cells, flattened cells, which eventually tend to form a monolayer, and small, ovoid, phase-dark cells with processes. The addition of vincristine, vindesine, or vinblastine at 0.02 μg/ml to these cultures results in a loss of processes and the swelling of the cell body of the small, phase-dark cells (Fig. 1). At this dose, all three compounds cause a reduction in the number of flattened cells compared with controls, presumably due to the antimitotic effects of these drugs [13]. This effect is not as pronounced at the lower doses.

The quantitative assay of relative toxicity consists in determining the number of the small cells that remain unaffected after 24 h exposure to the test compounds at varying dose levels. The dose reponse of the small, phase-dark cells in 3-day cultures to a 24-h exposure to vincristine, vindesine, and vinblastine is shown in Fig. 2. Analysis of variance of the means of two or three observations per dose in each of five different experiments indicates a significant difference among drugs ($P < 0.001$) and among doses ($P < 0.001$).

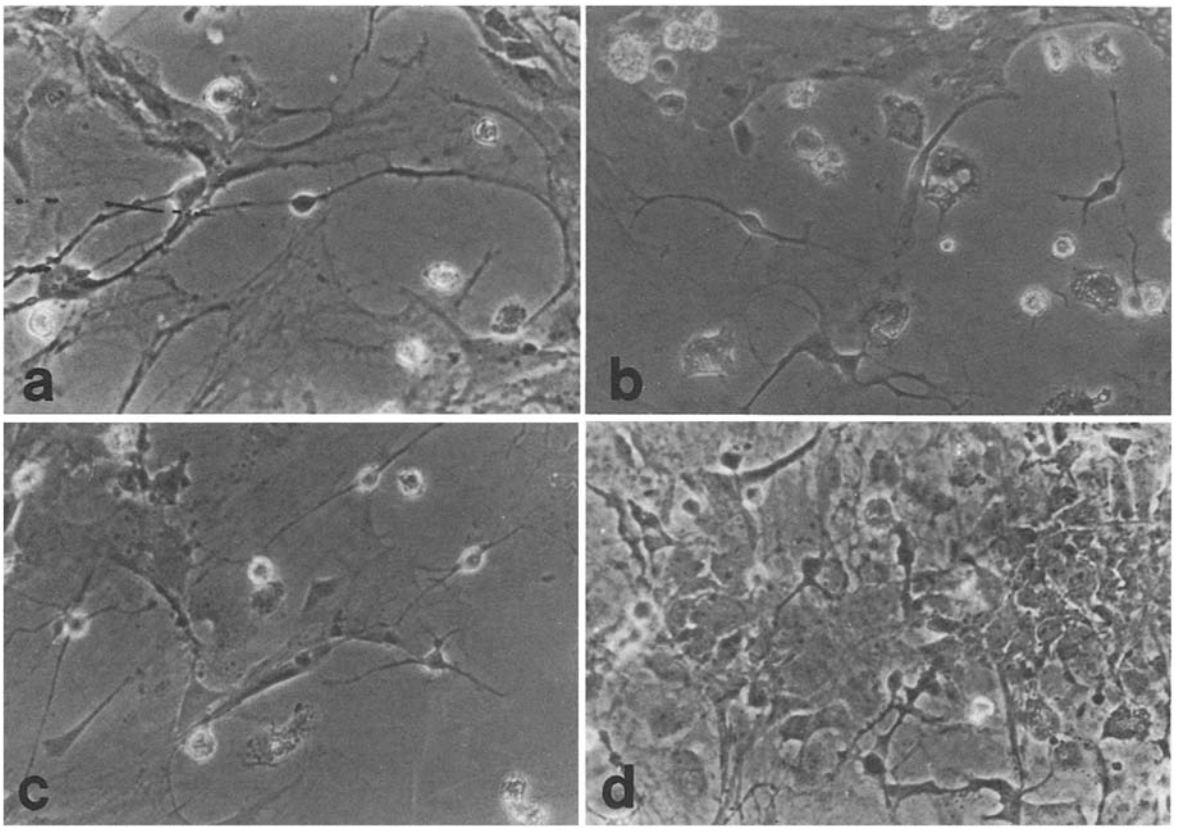

Fig. 3a—d. Recovery of cultured midbrain cells 5 days after a 24-h treatment with 0.02 μg/ml vincristine **a**, vindesine **b**, or vinblastine **c**. Control cells are also shown **d**. Phase contrast magnification × 216

Application of the Duncan Multiple Range Test indicates that each drug is significantly different from the others and from control at the $P=0.05$ level. From these observations the alkaloids may be listed in order of relative toxicity to cultured midbrain cells as: vincristine ≧ vindesine > vinblastine.

The ability of the small cells to recover was also examined. The medium was changed 24 h after drug addition. Five or 6 days later small cells with processes were found at all dose levels (Fig. 3), but the exact number present was difficult to determine because of the growth of the flat cells. At the higher doses some severely affected cells became detached during the period of drug treatment and were lost in the change of medium. Consequently, recovery would be expected to be incomplete at these doses.

Discussion

These results indicate that the relative toxicity of vincristine, vinblastine, and vindesine in the dissociated newborn rat midbrain in culture correlates with their relative neurotoxicity. The correlation is based on the number of small, phase-dark cells with processes that remain unaffected after 24 h of drug treatment. Whether these cells are glial or neuronal is difficult to determine on a morphologic basis, since cell morphology can be subject to extensive modulation by culture conditions [14]. Since Hirano and Zimmerman [6] have reported that neurons, astrocytes, oligodendroglia, and ependymal cells were all affected when vinblastine was implanted in the forebrains of rats, we feel that the identification of the type of cultured midbrain cell that responds to the vinca compounds is unnecessary for the purposes of the assay.

The differences in clinical neurotoxicity of vincristine, vinblastine, and vindesine may be due to pharmacokinetics and/or interaction with the microtubular and microfilamentous systems in cells. It has been reported [4] that vincristine, which is the most toxic, has the lowest body clearance; vinblastine, the least toxic, has the highest body clearance; and vindesine, which has an intermediate toxicity, also has an intermediate body clearance. The response of the cultured cells suggests, however, that the differential response of the nerve tissue itself may also be involved, and that this response is at least partially reversible. The mode of interaction of the vinca alkaloids with

neuronal and glial cells is unknown. Several light and electron microscopy studies have shown alterations resulting from vinca alkaloid treatment in the microtubules and neurofilaments in nerve tissue in vivo [7, 9, 12], and in vitro [2, 3, 10]. Vincristine, vinblastine and vindesine all block the in vitro polymerization of the microtubular protein, tubulin, but with equal effectiveness [5, 16].

Although pharmacokinetics has been precluded as a factor in the toxic response of the midbrain cells, there is still a possibility of differential uptake or metabolism of the vinca compounds, as well as microtubular and/or microfilamentous interaction. Studies on the structural determinants of the neurotoxic response are now in progress in our laboratory on cultured dissociated newborn rat superior cervical ganglion neurons.

In conclusion, the relative toxic response of dissociated newborn rat midbrain cells in culture to vincristine, vinblastine, and vindesine correlates with the relative clinical neurotoxicity of these vinca alkaloids, but the etiology of the interaction is unknown. To our knowledge, no such correlation has yet been reported for any other in vivo or in vitro model of vinca alkaloid neurotoxicity. Validation of the predictive elements of the cultured midbrain system now awaits clinical experience with novel vinca compounds.

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