Correlation of Cytotoxicity and Mitotic Spindle Dissolution by Vinblastine in Mammalian Cells

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SUMMARY

In two hamster cell lines that differed 100-fold in their vinblastine sensitivity, dissolution of the mitotic spindle by vinblastine in living cells correlated with cytotoxicity from vinblastine expressed as suppression of colony formation. The effect on the spindle apparatus occurred in 30 sec or less and thus provides a rapid assay for determining the cytotoxic effects of the Vinca alkaloids, as well as the potential for quantitative assay of solutions of Vinca derivatives.

INTRODUCTION

The mechanism by which the Vinca alkaloids VLB and VCR kill cells in vivo and in vitro has long been controversial (1, 3, 5, 9, 10). However, the interaction between the Vinca alkaloids and tubulin, a particular protein subunit of microtubules, has been extensively studied (2, 6, 7, 9, 10). VLB and VCR both have been shown to bind to tubulin with high affinity and relative specificity. Consequently, many authors have attributed cell death to mitotic arrest caused by tubulin binding by the Vinca alkaloids. Moreover, the concentration of VLB and VCR required to inhibit tubulin polymerization in vitro correlated with pharmacological concentrations of the drug in humans (8). At present, however, the interaction of Vinca alkaloids with tubulin in the mitotic spindle is not universally accepted as the mechanism of the chemotherapeutic effect of VLB or VCR in humans (4). The need therefore existed for a correlative set of experiments that related cellular cytotoxicity with tubulin-Vinca interaction. Using a pair of Chinese hamster cell lines with a 100-fold difference in cytotoxic sensitivity to VLB, we have studied the effects of varying concentrations of drug on the mitotic spindle of these living cells. We discovered that the dose response of the mitotic spindle dissolution correlated with that for the inhibition of colony formation and that the technology affords a rapid, reliable in vitro assay for determining the cytotoxic effect of the Vinca alkaloids.

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2 To whom requests for reprints should be addressed, at Sidney Farber Cancer Institute, 44 Binney Street, Boston, Mass. 02115.
3 The abbreviations used are: VLB, vinblastine; VCR, vincristine; DE10 (Grand Island Biological Co., Grand Island, N. Y.; No. H16). Cells were grown in 75-sq cm plastic flasks (Costar, Cambridge, Mass.; No. 3075) at 37° in 5% CO2-95% air. Collection of cells was performed by decanting the medium, rinsing the cell layer with PBS, pH 7.4 (Grand Island Biological), and then adding 2.5 ml of 0.25% trypsin in PBS. The cells were then incubated at room temperature until all cells were detached. DE10 was added to neutralize the trypsin, and the cell suspensions were used immediately in the assays of either colony formation or spindle birefringence.

Cell Culture. Two Chinese hamster fibroblast cell lines, B14-150 and B14-292, kindly supplied by Dr. Morgan Harris, University of California, were grown in DE10 (Grand Island Biological Co., Grand Island, N. Y.; No. H16). Cells were grown in 75-sq cm plastic flasks (Costar, Cambridge, Mass.; No. 3075) at 37° in 5% CO2-95% air. Collection of cells was performed by decanting the medium, rinsing the cell layer with PBS, pH 7.4 (Grand Island Biological), and then adding 2.5 ml of 0.25% trypsin in PBS. The cells were then incubated at room temperature until all cells were detached. DE10 was added to neutralize the trypsin, and the cell suspensions were used immediately in the assays of either colony formation or spindle birefringence.

RESULTS

Different concentrations of VLB from 1 × 10^-5 to 1 × 10^-9 M were added to cells in the wells in triplicate, and the colonies were counted after 7 days of incubation. Chart 1
VLB Cytotoxicity and Tubulin Binding

Chart 1. Effect of VLB treatment on mitotic spindle after 12 to 24 hr and on plating efficiency after 7 days. Cells from both cell lines were grown under sparse conditions in 3-ml wells for colony formation and on 12-mm glass coverslips for mitotic spindle birefringence measurements. After attachment and growth of the cells in each assay system for 4 to 12 hr, different concentrations of VLB were added. The number of colonies in the wells was counted after 7 days of incubation, and the number of birefringent mitotic spindles was counted after 12 to 24 hr. RMI, relative mitotic index (percentage of treated cells with normal mitotic spindles/percentage of untreated cells with normal mitotic spindles); RPE, relative plating efficiency (percentage of treated cells producing colonies/percentage of untreated cells producing colonies).

Illustrates the marked difference in cytotoxicity between the 2 cell lines. A 100-fold higher concentration of VLB was required to kill the B14-292 cells as compared to that required to kill the B14-150, with calculated ED₉₀ values of 8 × 10⁻⁹ M for the B14-150 line and of 9 × 10⁻⁷ M for the B14-292, similar to prior experiments with these cell lines (R. J. Owellen, unpublished observations).

In separate experiments, cells from both cell lines were also grown on strain-free coverslips in the absence and presence of varying concentrations of VLB. After 12 and 24 hr, the coverslips were examined in polarization microscopy for normal mitotic spindles per 100 cells. The relative mitotic index was calculated as shown in Chart 1. The ED₉₀ for spindle dissolution was 1 × 10⁻⁷ M for the 150 line, and 1 × 10⁻⁵ M for the 292 line, a 100-fold difference that parallels the cytotoxicity curves, although the latter were displaced downward by 8- to 9-fold at the ED₉₀. However, at the concentration that produces 100% inhibition of colony formation, cytotoxicity and spindle dissolution coincided. Therefore, the amount of VLB that produced a 50% fall in percentage of cells with mitotic spindles at 12 and 24 hr did not produce any change in plating efficiency at 7 days, but the VLB concentration that dissolved all mitotic spindles also completely inhibited plating efficiency. Moreover, perfusion or immersion of line B14-150 with 10⁻⁹ M VLB and line B14-292 with 10⁻⁷ M VLB both produced some abnormal (e.g., tripolar) spindles, and immersion in higher concentrations produced crystals (see Figs. 1 and 2 for each cell line). Moreover, Chart 2 and Fig. 3 illustrate that the perfusion of the cells in the chamber with VLB produced an immediate effect (within 30 sec) in the proportion of cells with normal mitotic spindles. In fact, the dose response of the relative mitotic index measured at 12 to 24 hr (Chart 1) was identical with that at 30 sec (Chart 2).

DISCUSSION

In 2 hamster cell lines that differed in their in vitro sensitivity to VLB, there was a close correlation between cytotoxicity (inhibition of colony formation) and dissolution of mitotic spindle. The VLB concentration that completely blocked the polymerization of tubulin into microtubules within 30 sec also completely prevented colony formation at 7 days. In a parallel manner, both the ED₉₀ of VLB for spindle dissolution and the ED₉₀ for cytotoxicity differed by 100-fold between the 2 cell lines, B14-150 and B14-292.

These experimental findings are consistent with the hypothesis that the mechanism by which VLB kills cells depends on the binding of VLB to tubulin. The possibility exists that other cellular events coincident with VLB binding to tubulin, and initiated within 30 sec after addition of VLB, may have killed the cells. From our present studies, however, the most economical conclusion is that VLB, and presumably all the other Vinca alkaloids, kills cells by binding to tubulin to cause the depolymerization of the...
mitotic spindle and other microtubules (2) in living cells. Disappearance of the mitotic spindle within 30 sec of VLB addition correlated with the complete absence of colony formation at 7 days, since absence of division connoted absence of growth. That a drop in the percentage of cells with a normal mitotic spindle to 50% of control in 24 hr did not change the number of colonies grown in the plates after 7 days suggests that the initial drug effect can be overcome with time or that this VLB concentration slowed growth rate, presumably producing the same number of colonies, only smaller in size (not measured). The crucial experimental fact is that the ED₅₀ and the concentration that causes 100% inhibition of colony formation were 100-fold higher for both cytotoxicity and mitotic spindle dissolution for cell line B14-292 as compared to line B14-150.

The fact that spindle dissolution in perfusion chambers over 30 sec correlated directly with cytotoxicity for these cells suggests that this technique may be a rapid and accurate method for defining the cytotoxic dose response of the Vinca alkaloids and other tubulin-binding drugs. This methodology will thus provide a rapid assay of drug-tubulin interaction in living cell systems as well as a rapid and accurate assay for new tubulin-binding drugs. In specialized cases, this technology may also provide quantitation of the concentrations of Vinca alkaloid and other tubulin-binding drugs in various fluid samples from patients.

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REFERENCES

Fig. 1. A, cell line 150, untreated. Mitotic spindle birefringence measured with λ/30 compensator was 1 to 1.5 nm. Bar, 25 μm. B, cell line 150, treated with 10^{-8} M VLB for 24 hr. Most spindles were abnormal, short, and stubby, and some cells had the appearance of abnormal collections of birefringent material. C, cell line 150, treated with 10^{-8} M VLB for 24 hr. No spindles apparent. D, cell line 150, treated with 10^{-4} M VLB for 24 hr. VLB crystals now appearing.
Fig. 2. A, cell line 292, untreated. Mitotic spindle identical with that of cell line 150. Bar, 25 µm. B, cell line 292, treated with 10⁻⁶ M VLB for 24 hr. Cultures had well-preserved mitotic spindles. The axes of the spindles illustrated here are not quite 45° to the axes of polarizer and analyzer, so that entire spindle is not well outlined. C, cell line 292, treated with 10⁻⁶ M VLB for 24 hr. No normal spindles; some abnormal tripolar spindles. D, cell line 292, treated with 10⁻⁶ M VLB for 24 hr. VLB crystal formation.
Fig. 3. A, cell line 150, untreated, mounted in perfusion chamber. Notice well-defined mitotic spindle near metaphase. B, same cell, 30 sec after perfusion with $10^{-7}$ M VLB. Notice complete disappearance of mitotic spindle and formation of cytoplasmic blebs.
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