Effects of Microtubule Inhibitors on Etoposide Accumulation and DNA Damage in Human K562 Cells in Vitro

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ABSTRACT

The effects of microtubule inhibitors on cellular accumulation of 4'-demethyllepoipodophyllotoxin-9-(4,6-O-ethylidene-b-D-glucopyranoside) (VP-16) and subsequent epipodophyllotoxin-induced DNA single-strand breaks were investigated in human leukemia K562 cells. At concentrations of 0.05–20 μM, vinblastine, vincristine, and maytansine similarly increased the steady-state cell concentration of VP-16 (2.5 μM) up to 2-fold. Following removal of extracellular vinblastine, the elevation of cell VP-16 was maintained through an additional 55-min incubation period. Washing cells free of extracellular VP-16 resulted in a nonexchangeable (or bound) component comprising 15–17% of the VP-16 concentration found before removal of extracellular drug. In cells incubated with VP-16 alone, removal of extracellular drug resulted in less than 5% cell retention of drug. At vinblastine concentrations of 0.05–0.2 μM, the increase in cell VP-16 was due to a progressive increase in nonexchangeable VP-16. At greater vinblastine concentrations, up to 10 μM, there was no further increase in nonexchangeable VP-16 but there was a 1.6-fold increase in the exchangeable (or free) concentration of VP-16. Similar elevation of both nonexchangeable and exchangeable VP-16 by 10 μM vincristine and maytansine was observed; however, 50–100 μM podophyllotoxin or taxol was required for comparable elevation of exchangeable drug with no increase of nonexchangeable VP-16. Elevation of the calcium chelator EGTA on VP-16 in the presence of vincristine was due to inhibition of the unidirectional efflux of this epipodophyllotoxin with a 69% decline in the rate constant for efflux. There were no effects of vinblastine on VP-16 influx. There was no enhancement of DNA single-strand break frequency when cells were incubated with 2.5 μM VP-16 and 0.2 μM vinblastine, a concentration of the Vinca alkaloid that increased only nonexchangeable VP-16. VP-16-induced DNA damage was enhanced by vinblastine concentrations above 0.5 μM, concentrations that elevated exchangeable VP-16, with a maximum doubling of radiation equivalent single-strand break frequency observed with 20 μM vinblastine, consistent with the maximum elevation of cell VP-16 with 20 μM Vinca alkaloid. These results indicate that vinblastine and other microtubule inhibitors elevate cell VP-16 by inhibition of the efflux of exchangeable drug and by increasing the level of nonexchangeable drug. Potentiation of VP-16-induced DNA damage is observed only at microtubule inhibitor concentrations which elevate extracellular VP-16. Studies are under way to identify the cellular locus of elevated nonexchangeable VP-16 and to characterize the cytotoxic role of this bound component.

INTRODUCTION

Unlike Vinca alkaloids and anthracyclines, the epipodophyllotoxin VP-16 is poorly retained within cells and is therefore a good prototype for the study of modulation of drug transport across the cell membrane, subsequent effects on cell disposition of drug, and cytotoxicity. Previous reports by this author (1, 2) demonstrated that the calcium antagonist verapamil enhances the cellular concentration and activity of VP-16 by inhibition of drug efflux. These findings are consistent with the suggestion that modulating agents such as calcium antagonists elevate cell concentrations of natural product agents by inhibition of efflux perhaps by competition for the same membrane carrier system, especially in those cells which are multiply drug resistant due to reduced accumulation of drug (3–5). It has been suggested that semisynthetic and natural product anticancer agents such as the epipodophyllotoxins, Vinca alkaloids, and anthracyclines share a common efflux system, hence the pattern of cross-resistance in a number of cell lines selected for resistance with any one of the classes of antineoplastics (5–10). The present report of the effects of microtubule inhibitors on VP-16 accumulation and subsequent activity was prompted in part by the possibility that Vinca alkaloids compete with VP-16 for efflux from the cell. Also, microtubule inhibitors such as vincristine and vinblastine have been shown to inhibit membrane transport processes linked to cellular energy metabolism (11, 12). For example, vincristine inhibits the energy-dependent efflux of methotrexate resulting in elevated drug concentration and activity of the antifolate (12). Since there is some evidence that epipodophyllotoxin efflux is associated with cellular energy metabolism (2, 13, 14), there is reason to believe that microtubule inhibitors similarly affect VP-16 accumulation by inhibition of efflux.

Interest in the microtubule inhibitor-epipodophyllotoxin combination in vitro was stimulated also by previous reports of the effectiveness of the Vinca alkaloid-epipodophyllotoxin combination in vivo. Jackson et al. (15, 16) demonstrated therapeutic synergism in L1210-bearing mice when vincristine was combined with VP-16. Also, in children with acute lymphoblastic leukemia who have relapsed after end of therapy, VP-16 and VM-26 have been used in combination with vincristine to overcome clinical resistance to the Vinca alkaloid (17, 18). The in vitro cellular studies reported here provide a possible explanation for the effective clinical combination of microtubule inhibitors and epipodophyllotoxins and serve as the basis for further examination of this potentially important drug combination.

MATERIALS AND METHODS

Chemicals. [3H]VP-16 was obtained from Moravek Biochemicals (Brea, CA). The radiochemical purity of the [3H]VP-16 was 92–97%, as measured by high-performance liquid chromatography (19). All VP-16 solutions were prepared in either dimethyl sulfoxide or 50% methanol. Solvent concentrations did not exceed 0.6% in the culture medium after drug treatment and were present in control flasks at equivalent levels. [2-14C]Thymidine (53 mCi/mmol) and [methyl-3H]thymidine (20 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Verapamil was provided by Knoll Pharmaceuticals (Whippany, NJ). VP-16 was provided by Bristol Laboratories (Syracuse, NY). Maytansine and taxol were obtained from the Drug Investigational Branch of the National Cancer Institute. Vincristine, vinblastine, colchicine, and protease K were obtained from Sigma (St. Louis, MO). Cell culture medium and fetal calf serum were purchased from K. C. Biological (Ardsdale, NY).

Cells, Media, and Incubation Techniques. Human leukemia K562
The intracellular water volume was determined to be 4.12 ± 0.10 ml/g content expressed as nmol/g dry weight. Intracellular water/g dry media and the absence of a concentrative mechanism for cell VP-16 to cell suspension was found associated with cells at a cell density of 1 x 10^6 cells/ml consistent with the lack of substrate depletion from the media and the absence of a concentrative mechanism for cell VP-16 accumulation.

VP-16-mediated DNA damage was assessed using the alkaline elution technique for high-frequency single-strand breaks (20). Intact K562 cells previously labeled with [2-^3H]thymidine were suspended at a density of 5 x 10^6 cells/ml in a pH 7.4 buffer (buffer A) of NaCl (110 mM), KCl (5 mM), MgCl_2 (1 mM), NaH_2PO_4 (5 mM), (2-hydroxyethyl)-1-piperazineethanesulfonic acid (25 mM), and glucose (10 mM). These cells were incubated with 2.5 μM VP-16 at 37°C for 20 min in the presence or absence of various microtubule inhibitors. L1210 cells (5 x 10^6) containing [3H]DNA which had received 1500 rad irradiation were added as internal standards to 7.5 x 10^6 drug-treated K562 cells containing [4^C]DNA. After two washings in cold buffer A, cells were layered onto a polyvinyl chloride filter (pore size, 2 μm; Millipore Corp., Bedford, MA) and lysed with a solution of 2% sodium dodecyl sulfate, 10 mM disodium EDTA, and protease K (0.5 mg/ml). The DNA was eluted from the filter with tetrapropylammonium hydroxide, pH 12.1. The elution flow rate was 0.16 to 0.20 ml/min, with a fractional interval of 5 min and a total elution time of 30 min. Cells containing [3H]DNA were irradiated on ice with a 127Cs source (Mark Irradiator; J. L. Sheppard and Associates, Glendale, CA) using an exposure rate of 842 rads/min. The frequency of VP-16-induced DNA SSBs was quantitated as the fraction of [4^C]DNA remaining on the filter, under a variety of experimental conditions, when 75% of the ^3H-labeled internal standard DNA remains. A calibration curve for relating the frequency of VP-16-induced DNA SSBs to a corresponding effect of radiation (radiation equivalent DNA damage) using [4^C]-labeled cells was obtained by plotting rads versus [4^C]DNA retention at 75% retention of the [3H]DNA internal standard. The radiation equivalent DNA damage induced by VP-16 alone was compared with that seen when various microtubule inhibitors were combined with VP-16. Data are expressed as percentage of control radiation equivalent DNA damage.

Drug Accumulation. K562 cells were suspended in buffer A at a final concentration of 0.5-1 x 10^6 cells/ml. Cells were stirred in specially designed flasks by revolving Teflon paddles in a 37°C water bath, as described previously (21). For determination of cellular [3H]VP-16 uptake, 1-ml portions of cell suspension containing [3H]epipodophyllotoxin were periodically injected into 10 volumes of 0.85% NaCl solution at 0°C. Cell fractions were then separated by centrifugation (500 x g for 2 min) and washed twice with 0.85% NaCl solution at 0°C. The washed pellet was drawn up into a plastic pipet tip, extruded onto a polyethylene tare, and dried overnight at 70°C. The dried pellets were weighed, placed in a glass scintillation vial, and dissolved in 0.25 ml of 1 N KOH for 90 min at 70°C. The digest was neutralized with 0.25 ml of 1 N HCl; 4 ml of aqueous counting scintillant (Amersham Corp., Arlington Heights, IL) were added, and radioactivity was determined on a liquid scintillation spectrometer. Counting efficiencies were determined using [3H]toluene standards. Results yield cellular drug content expressed as nmol/g dry weight. Intracellular water/g dry weight was determined from the difference between the wet and dry weights of cell pellets, less the [4^C]lucinulin space as described elsewhere (22). Molar intracellular drug concentration was then determined from the molar content of cell VP-16 and the intracellular H_2O volume. At 2.5 μM extracellular [3H]VP-16, exchangeable intracellular concentrations attained were 12.1 ± 0.3 nmol/g dry weight which is equivalent to 2.8 μM and an intracellular/extracellular concentration ratio of 1.1. The intracellular water volume was determined to be 4.12 ± 0.10 ml/g dry weight of K562 cells. Less than 0.7% of total radioactivity added to cell suspension was found associated with cells at a cell density of 1 x 10^6 cells/ml consistent with the lack of substrate depletion from the media and the absence of a concentrative mechanism for cell VP-16 accumulation.

RESULTS

Effects of Vinblastine on Accumulation of VP-16 in K562 Cells. Fig. 1 indicates that vinblastine concentrations greater than 20 nM enhanced the uptake of VP-16 in a time- and concentration-dependent manner with maximum effect at 10-20 μM. Preincubation with vinblastine for up to 20 min prior to VP-16 addition did not alter the profile shown in Fig. 1. The rapid onset of vinblastine-induced increase of VP-16 concentration is indicated in Fig. 2, where the Vinca alkaloid was added after a steady-state epipodophyllotoxin concentration was achieved. Vincristine (not shown) or the microtubule inhibitor maytansine was combined with [3H]VP-16 under the same conditions as those shown in Figs. 1 and 2. The enhancement of VP-16 concentrations under these conditions was similar in magnitude and time of onset to those observed for vinblastine. No effect on cell VP-16 was observed when 25 μM Adriamycin was added.

The persistence of vinblastine enhancement of VP-16 after removal of extracellular Vinca alkaloid is illustrated in Fig. 3. Cells were incubated with 2.5 μM [3H]VP-16 alone or together with 0.2 or 10 μM vinblastine over a total incubation period of more than 50 min. After 20 min (Fig. 3, arrows), portions of the cell suspensions were washed free of vinblastine and resuspended in fresh buffer at 37°C containing the original 2.5 μM [3H]VP-16. Subsequent quantitation of cell VP-16 (Fig. 3, 50 60 Minutes

![Fig. 1](image1.png) Fig. 1. Effect of various concentrations of vinblastine (VLB) on uptake of VP-16 in K562 cells. Cells were incubated for 30 s with vinblastine after which 2.5 μM [3H]VP-16 was added and accumulation of drug was measured over the next 55 min.

![Fig. 2](image2.png) Fig. 2. Effects of Adriamycin (25 μM), maytansine (0.2 μM), and vinblastine (0.2 and 20 μM) on net accumulation of VP-16 in K562 cells. Cells were brought to steady state with 2.5 μM [3H]VP-16. At arrow, portions of the cell suspension were transferred to flasks containing the various agents.
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Fig. 3. Effects of an initial 20-min incubation of K562 cells with vinblastine (VLB) on subsequent cellular levels of VP-16. Cells were incubated with 2.5 μM [3H]VP-16 alone (control) or in the presence of 0.2 or 10 μM vinblastine. At 20 min (arrows) a portion of cell suspension from each flask was washed free of all drugs and resuspended in the original 2.5 μM [3H]VP-16. Cell VP-16 was measured over the next 35 min; closed symbols correspond to the original incubation conditions after removal of vinblastine in the continued presence of [3H]VP-16.

Fig. 4. Effect of vinblastine (VLB) on exchangeable and nonexchangeable cell VP-16 in K562 cells. Cells were incubated with 2.5 μM [3H]VP-16 alone (control) or together with 10 μM vinblastine (VLB). At 20 min (left arrows), cells were washed free of all drugs and resuspended in fresh buffer at 37°C. Over the next 40 min, the intracellular VP-16 level was measured. At 62 min, a portion of cells from each flask was again washed and resuspended in fresh buffer at 37°C (right arrows). Cell VP-16 was measured (●, ●) over the next 30 min.

closed symbols) indicates no alteration of epipodophyllotoxin in control cells due to washing and resuspension and a parallel, enhanced accumulation of VP-16 in cells whether or not vinblastine was continuously present. In similar experiments, prolonged elevation of VP-16 levels after a brief exposure (20 min) to 0.2 μM vincristine has been observed through at least 55 min after removal of the Vinca alkaloid. In contrast, continuous exposure of K562 cells to the calcium antagonist verapamil is required for sustained enhancement of cell VP-16 (not shown), consistent with the rapid reversibility of verapamil effects on VP-16 accumulation and activity in L1210 cells (2). In separate experiments, cells which were incubated with 5 μM [3H]VP-16 to a steady-state concentration and subsequently resuspended in 2.5 μM [3H]VP-16 rapidly lost drug back to levels comparable to that seen when cells were originally incubated with 2.5 μM [3H]VP-16 (not shown). These results indicate that prolonged vinblastine effects on VP-16 accumulation are not the consequence of an initial elevation of cell epipodophyllotoxin.

Nonexchangeable VP-16 was quantitated by washing cells free of all extracellular drugs after a 20-min incubation with 10 μM vinblastine and 2.5 μM [3H]VP-16 (Fig. 4). Characteristic of VP-16, less than 5% of cellular drug was retained under control conditions. However, initial incubation with vinblastine resulted not only in a large increase in total cell VP-16 but also in a significant increase in the nonexchangeable component which was maintained even after a second washing and resuspension in drug-free buffer. Addition of 100 μM unlabeled VP-16 during efflux did not alter the nonexchangeable component. In addition, high-performance liquid chromatographic analysis of nonexchangeable VP-16 extracted from cells indicated that greater than 92% of radioactivity was present as intact [3H]VP-16. These data demonstrate that 10 μM vinblastine increases cell VP-16 by increasing bound and free levels of this epipodophyllotoxin.

Effects of Vinblastine on the Initial Uptake and Efflux of VP-16. Since elevation of nonexchangeable VP-16 accounted for only a portion of the total vinblastine-mediated increase of cell VP-16 [12 nmol/g dry weight increase in total VP-16 after 20 min versus 4.5 nmol/g dry weight increase in nonexchangeable VP-16 (Fig. 4)], the effects of vinblastine on the bidirectional fluxes of VP-16 across the plasma membrane were examined. Vinblastine (10 μM), added simultaneously with or 20 min prior to [3H]VP-16, had no effect on initial uptake of [3H]VP-16 (2.5–10 μM) measured every 10 s over a 100-s time course (not shown). In addition, as demonstrated previously in LI210 cells (2), uptake of VP-16 over this interval represents initial influx with little cell surface binding since the time course for uptake can be extrapolated back to zero time with no appreciable accumulation of [3H]VP-16.

Fig. 5 illustrates the vinblastine-mediated inhibition of the rate of cellular loss of VP-16. Cells were loaded to the same level of VP-16 by incubation for 20 min with 8.75 μM [3H]VP-16 alone or 5 μM [3H]VP-16 in the presence of 10 μM vinblastine. Cells were then washed free of drugs and resuspended into VP-16-free buffer, with or without 10 μM vinblastine as before. Upon efflux of VP-16 in the presence of vinblastine there was an evident increase in the nonexchangeable component compared to controls. In Fig. 5 (inset), the fraction of exchangeable cell VP-16 is plotted as a function of time after resuspension into VP-16-free buffer in control and vinblastine-treated cells. Averaging 5 experiments run on separate days, there was a 68.6 ± 5.6% (SE) decline in the unidirectional rate constant for efflux.

Time and Concentration Dependence of Microtubule Inhibitor-induced Enhancement of Nonexchangeable VP-16. Fig. 6 illustrates the accumulation and subsequent retention of VP-16 in the presence of various concentrations of vinblastine. Cells were incubated for 20 min with 2.5 μM [3H]VP-16 and the indicated concentration of vinblastine, and total cell epipodophyllotoxin was determined. Nonexchangeable VP-16 was measured after cells were washed twice, resuspended in drug-free buffer, and incubated for an additional 40 min. Under control conditions, 5% of total accumulated VP-16 was retained after efflux. As the vinblastine concentration was increased from 0.05 to 10 μM there was a progressive increase in total accumulated VP-16. There was also an increase in the nonexchangeable level of epipodophyllotoxin as vinblastine concentration was increased from 0.05 to 0.2 μM. As vinblastine concentrations were increased further, there was no additional increase in the nonexchangeable component of VP-16. In separate experiments, cells were incubated with 2.5 μM [3H]VP-16 and 10 μM vinblastine for up to 1 h; total and nonexchangeable cell VP-16 was determined at various intervals during this incubation period. Beyond 20 min, there was no further increase in the nonexchangeable concentration of VP-16 (not shown). At the clinically relevant concentrations of vinblastine (0.05 and 0.2 μM) (23, 24), the increase of total cell VP-16 could be accounted for
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Fig. 5. Effect of vinblastine (VLB) on VP-16 efflux in K562 cells. Cells were incubated for 20 min to achieve the same level of VP-16 by incubation with 5 mM [3H]VP-16 plus 16 

Cellular epipodophyllotoxin level was measured, and the cells were re suspended in twice the volume of VP-16-free media. Vinblastine-treated cells were again exposed to 10 mM vinblastine. Over the next 35 min, the intracellular epipodophyllotoxin level was measured. Inset, log of the fraction of exchangeable epipodophyllotoxin remaining in the cell as a function of time after resuspension into VP-16-free buffer. Data are representative of 5 similar experiments used to calculate the unidirectional rate constants for efflux cited in the text.

Fig. 6. Increased accumulation and retention of VP-16 in K562 cells in the presence of vinblastine. Cells were incubated for 20 min with 2.5 mM [3H]VP-16 alone or together with the indicated concentrations of vinblastine. Total cell VP-16 was measured in triplicate. Cells were subsequently washed twice to remove extracellular drug and resuspended in drug-free buffer. The nonexchangeable fraction of cell VP-16 remaining after 40 min incubation in drug-free buffer was measured in triplicate and is shown in the shaded area. Columns, mean for 3-38 separate experiments run on separate days. Bars, SE.

by an increase in the nonexchangeable component (Fig. 6; Table 1). Similarly, 0.2 mM vincristine and 0.2 mM maytansine, a microtubule inhibitor known to compete with vinblastine for binding to tubulin (25), increased the nonexchangeable component of VP-16, and to the same extent as that observed with 0.2 mM vinblastine (not shown). At higher concentrations of these microtubule inhibitors (10 

Table 1  Effects of microtubule inhibitors and verapamil on cellular levels of VP-16

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Cell VP-16 (nmol/g dry wt)</th>
<th>Total</th>
<th>Nonexchangeable</th>
<th>Exchangeable</th>
<th>% of control exchangeable</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP-16 (2.5 mM)</td>
<td>12.84 ± 0.33 (38)*</td>
<td>0.75 ± 0.05 (20)</td>
<td>12.09</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
| + 0.2 


* Cells were incubated with 2.5 mM [3H]VP-16 alone or together with the indicated agents for 20 min, following which samples were taken for analysis of total [3H]epipodophyllotoxin. Nonexchangeable VP-16 levels were determined in triplicate after cells were incubated in VP-16-free buffer for 40 min. Exchangeable epipodophyllotoxin was calculated by taking the difference of total and nonexchangeable drug.

** Mean ± SE; numbers in parentheses, number of experiments performed on different days.
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The present study demonstrates that a variety of microtubule inhibitors enhance the accumulation of VP-16 by slowing the loss of the epipodophyllotoxin from cells. Intracellular accumulation of [3H]VP-16 was enhanced at vinblastine concentrations above 20 nM (Fig. 1), indicating that this modulation occurs at clinically achievable concentrations for the vincas (23, 24). The onset and magnitude of microtubule inhibitor-mediated enhancement of VP-16 accumulation were rapid (2–5 min) over a range of concentrations whether the modulating agent was added simultaneously with [3H]VP-16 (Fig. 1) or after a steady-state concentration of epipodophyllotoxin had been achieved (Fig. 2). Interestingly, Adriamycin had no effect on VP-16 accumulation even when present at 10-fold the extracellular concentration of the epipodophyllotoxin (Fig. 2). This result does not support the suggestion that these agents share a common membrane carrier system (14).

The Vinca alkaloid-mediated enhancement of cell VP-16 accumulation is not reversible for at least 1 h after removal of either vinblastine or vincristine (Fig. 3). These results suggest that at drug concentrations achieved clinically, the elevation of cell VP-16 concentration by microtubule inhibitors may be maintained for a sufficient period, after only a brief exposure to Vinca alkaloids, to derive the therapeutic synergism observed in vivo to animals (15, 16) and the effective response reported clinically (17, 18). Experiments are under way to investigate, over a more prolonged incubation period, the duration of enhancement of VP-16 after preincubation with microtubule inhibitors, in order to better understand the relationship between intracellular microtubule inhibitor concentration and the effect on VP-16 accumulation and cell disposition.

In a previous report by Allen (13), vinblastine at 100 μM did not affect steady-state cell accumulation of VP-16 or VM-26 when these agents were incubated with L1210 cells at an extracellular concentration of 15 μM. Although human leukemia K562 cells were used in the present report, Vinca alkaloid-mediated elevation of VP-16 (used at an extracellular concentration of 5 μM) in L1210 cells has been reported (32). Allen (13) suggested that vinblastine altered the proportion of tightly bound drug to exchangeable drug without affecting the steady-state accumulation of the epipodophyllotoxin. This effect was seen at 3 μM vinblastine with the nonexchangeable drug component elevated by 50% over controls compared to the 3–4-fold elevation observed in the present report at comparable Vinca alkaloid concentrations (Table 1).

Vincristine and maytansine, which compete with vinblastine for binding to tubulin heterodimers (25), were found to be at least as potent as vinblastine in enhancing cell VP-16 accumulation, via elevation of exchangeable and nonexchangeable epipodophyllotoxin (Table 1). In contrast, podophyllotoxin, the aglycone parent compound for VP-16, does not promote binding of VP-16 within the cell (Table 1). Podophyllotoxin, which binds to tubulin at a separate site from the Vinca alkaloids (26, 28), is much less potent than vincristine, vinblastine, and maytansine in elevating the concentration of exchangeable VP-16

![DNA single-strand breaks in K562 cells treated for 20 min in the presence of VP-16 (2.5 μM) alone or together with 0.2 or 10 μM vinblastine. The fraction of retained [3H]DNA from experimental cells is plotted against the fraction of retained [3H]labeled internal standard DNA. The internal standard cells were irradiated with 1500 rads. The DNA was eluted at pH 12.1.](image)

Table 2 Vinblastine effects on VP-16-induced SSB in K562 cells

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>SSB (rad equivalents)</th>
<th>% of control VP-16 SSB</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP-16 (2.5 μM)</td>
<td>1029 ± 139 (6)</td>
<td>100</td>
</tr>
<tr>
<td>+ 0.5 μM vinblastine</td>
<td>1000 ± 119 (7)</td>
<td>97</td>
</tr>
<tr>
<td>+ 2 μM vinblastine</td>
<td>1314 ± 132 (5)</td>
<td>128</td>
</tr>
<tr>
<td>+ 10 μM vinblastine</td>
<td>1930 ± 227 (8)</td>
<td>188</td>
</tr>
<tr>
<td>+ 20 μM vinblastine</td>
<td>2393 ± 250 (4)</td>
<td>233</td>
</tr>
<tr>
<td>+ 50 μM vinblastine</td>
<td>2377 ± 348 (4)</td>
<td>231</td>
</tr>
<tr>
<td>+ 10 μM vincristine</td>
<td>1681 ± 237 (5)</td>
<td>163</td>
</tr>
<tr>
<td>+ 10 μM maytansine</td>
<td>1743 ± 323 (4)</td>
<td>169</td>
</tr>
<tr>
<td>+ 100 mM podophyllotoxin</td>
<td>1483 ± 223 (3)</td>
<td>144</td>
</tr>
<tr>
<td>+ 100 mM taxol</td>
<td>1659 ± 239 (3)</td>
<td>161</td>
</tr>
<tr>
<td>+ 100 mM colchicine</td>
<td>1072 ± 256 (3)</td>
<td>104</td>
</tr>
</tbody>
</table>

* All drug treatments were for 20 min.

* Rad equivalents were determined as described in “Materials and Methods.”

* Mean ± SE; numbers in parentheses, number of experiments performed on different days.

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within cells (Table 1). At 10 μM podophyllotoxin, there is no effect on VP-16 accumulation, whereas at 100 μM podophyllotoxin, the rate constant for VP-16 efflux is inhibited by 45% (not shown) compared to 69% for 10 μM vinblastine. The microtubule inhibitor taxol, which binds to tubulin at a separate site from both the Vinca alkaloids and podophyllotoxin (27), similarly does not elevate nonexchangeable VP-16 levels even at the high concentration (100 μM) which causes enhancement of exchangeable epipodophyllotoxin (Table 1). Hence, only microtubule inhibitors which compete for tubulin binding at the so-called “vinblastine binding site” increase nonexchangeable VP-16.

The progressive elevation of nonexchangeable VP-16 in the presence of vinblastine at the lower concentrations of the Vinca alkaloid used (0.05 and 0.2 μM) accounted for the increase of cell VP-16 concentration (Table 1). As the Vinca alkaloid concentration was increased there was no further increase in the nonexchangeable component but there was, in addition, a progressive increase in the exchangeable component of cell VP-16 (Fig. 6; Table 1). These data suggest that, at the clinically relevant submicromolar concentrations of the Vinca alkaloids, nonexchangeable VP-16 may be increased during short incubation periods and might correlate with therapeutic response. The elevation of nonexchangeable VP-16 was not associated with enhancement of VP-16-induced SSB in DNA (compare Table 1 and Table 2). Since VP-16 cytotoxicity has been correlated with DNA single- and double-strand breaks as an example of the enhancement of DNA damage by calcium antagonists in 1,1210 cells in vitro. Cancer Res., 44: 3360-3365, 1984.


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