Differential Activity of Vincristine and Vinblastine against Cultured Cells

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ABSTRACT

Vincristine and vinblastine exhibit differential activity against tumors and normal tissues. In this work, a number of cultured cell lines were assayed for their sensitivity to the antiproliferative and cytotoxic effects of the two drugs following short-term (4 hr) or during continuous exposures. Differential activity was not seen when cells were subjected to continuous exposures. The concentrations of vincristine and vinblastine, respectively, that inhibited growth rates by 50% were: mouse leukemia L1210 cells, 4.4 and 4.0 nM; mouse lymphoma S49 cells, 5 and 3.5 nM; mouse neuroblastoma cells, 33 and 15 nM; HeLa cells, 1.4 and 2.6 nM; and human leukemia HL-60 cells, 4.1 and 5.3 nM. In contrast, differential toxicity was seen when cells were subjected to 4-hr exposures and transferred to drug-free medium: the 50% growth-inhibitory concentrations for vincristine and vinblastine, respectively, for inhibition (a) of proliferation of L1210 cells were 100 and 380 nM and of HL-60 cells were 23 and 900 nM and (b) of colony formation of L1210 cells were 6 and >600 nM and of HeLa cells were 33 and 62 nM. Uptake and release of [3H]-vincristine and [3H]vinblastine were examined in L1210 cells under the conditions of growth experiments. Uptake of both drugs was dependent on the pH of culture media, and significantly greater amounts of [3H]vinblastine than of [3H]vincristine were associated with cells after 4-hr exposures to equal concentrations of either drug. When cells were transferred to drug-free medium after 4-hr exposures, vinblastine was released much more rapidly from cells than was vincristine, and by 0.5 hr after resuspension of cells, the amount of vincristine associated with the cells was greater than the amount of vinblastine and remained so for up to at least 6 hr.

INTRODUCTION

Vincristine and vinblastine are potent mitotic inhibitors that have been used clinically in the treatment of a variety of neoplasms. Although they differ in their molecular structure by only one carbonyl group, vinblastine and vincristine exhibit quite different spectra of activity in both their targets of antitumor activity and their sites of dose-limiting toxicity. The major sites of toxicity for vincristine and vinblastine, respectively, are the nervous system and the hematopoietic system (1).

The objectives of the current study were to identify cell types that differ in their sensitivity to vincristine and vinblastine and to determine if differential activity is related to differences in uptake or release of drug. Included were cells from these established lines: mouse neuroblastoma; mouse leukemia L1210; mouse lymphoma S49; HeLa; and human promyelocytic leukemia HL-60. Drug sensitivity in each cell line was determined by assaying inhibition of proliferation during a continuous exposure to either drug or by assaying colony formation following a 24-hr exposure. Because of rapid loss of Vinca alkaloids from human serum following an i.v. bolus injection (11, 12, 14, 17), the sensitivity of cells to short-term (1- and 4-hr) exposures was also determined for both drugs. Although there were differences in sensitivity to vincristine and vinblastine between cell lines, there was little or no difference in sensitivity to the 2 drugs within a given cell line during continuous exposures. In contrast, after exposures of 4 hr or less, L1210 and HL-60 cells were much more sensitive to vincristine than to vinblastine. Experiments were undertaken to determine if the difference in sensitivity of L1210 cells to vincristine and vinblastine after short exposures was due to differences in uptake and/or release of the drugs by cells. Others have observed that vinblastine associates with and is released more quickly from rat platelets than vincristine (8). Radiolabeled vincristine and vinblastine were utilized in assaying uptake and release of drugs from the cells during and following 4-hr exposures. The results obtained suggest that rapid release of vinblastine by cells is the reason for its lesser toxicity, compared with vincristine, after 1- or 4-hr exposures.

MATERIALS AND METHODS

Cell Culture

Unless otherwise noted, stock cultures of the cell lines described below were restarted after 30 to 40 subculture generations from frozen cells that had been demonstrated to be free of Mycoplasma (Dr. J. Robertson, Medical Bacteriology, University of Alberta). Stock cultures were routinely maintained in antibiotic-free growth medium of the appropriate type and were expanded in antibiotic-containing medium (penicillin, 100 units/ml; streptomycin, 100 µg/ml; and, in some cases, gentamycin, 50 µg/ml) to obtain cells for experimental use. All cultures were grown at 37° in 5% CO2 in air, and cell numbers were determined by enumeration with an electronic particle counter (Coulter Electronics, Hialeah, FL).

L1210 Cells. The origin and characteristics of mouse leukemia L1210/C2 cells have been described (2). Stock cultures were routinely maintained in Fischer’s medium with 10% horse serum, and 5 mM HEPES and antibiotics were added to the growth medium of experimental cultures. Cells grew exponentially with doubling times of 14 to 16 hr.

1 Supported by the National Cancer Institute of Canada and the Alberta Heritage Savings Trust Fund: Applied Research—Cancer. Portions of this work were presented in a preliminary report (7).
2 Recipient of a Studentship Award from the Alberta Heritage Foundation for Medical Research.
3 To whom requests for reprints should be addressed.
Received August 22, 1983; accepted May 8, 1984.

4 Uptake refers to total cell-associated drug and may include drug adsorbed to cell surfaces as well as internalized drug.
5 The abbreviations used are: HEPES, N-2-hydroxyethylpiperazine-N' -2-ethanesulfonate; IC50, concentration of drug that inhibited proliferation rates by 50% over the period of time specified in text; IC50, concentration of drug that gave 10% survival.

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The effects of drugs on proliferation and viability of L1210/C2 cells after 4- or 24-hr exposures were determined as described previously (2). S49 Cells. Mouse lymphoma S49 cells were provided by Dr. D. W. Martin, Jr., University of California, San Francisco, CA, and were maintained in the same manner as L1210 cells. Drug effects on cell proliferation were also determined as described for L1210 cells (2). Cells grew exponentially with doubling times of about 15 hr.

HL-60 Cells. Human promyelocytic leukemia HL-60 cells (6) were a generous gift from Dr. R. Gallo, NIH, Bethesda, MD. Growth medium consisted of Roswell Park Memorial Institute Medium 1640 supplemented with 15% fetal calf serum. Stock cultures were subcultured 3 or more times per week by diluting cells to 10^6/ml. Such cells grew exponentially with doubling times of about 36 hr. Drug effects on proliferation of HL-60 cells were determined by a modification of the procedure described previously (2) for L1210 cells. Drug exposures were initiated by combining suspensions of exponentially proliferating cells (2 x 10^5 cells/ml) with equal volumes of fresh growth medium containing drug at twice the concentration to be tested. Duplicate cultures were prepared for each drug concentration. For exposures of 4 hr, cells were removed from the drug solution by centrifugation, washed, and resuspended in the original volume of drug-free growth medium. Cell recovery was about 85 to 90% for all cultures including controls.

HeLa Cells. Maintenance of stock cultures of HeLa S3 cells and expansion of cells for experimental use were carried out as described previously (3). In suspension cultures, cell concentrations were maintained below 5 x 10^5 cells/ml, and cell proliferation was exponential with doubling times of about 20 hr. The effects of drugs on the proliferation rate of HeLa cells were evaluated by determining cell numbers in drug-treated monolayer cultures during a 48-hr period as follows. One-ounce prescription bottles (Brockway Glass Co., Brockway, PA) were conditioned for 24 hr at 37° with Eagle's minimal essential medium for suspension culture supplemented with 20% calf serum and with antibiotics, a procedure shown previously to increase plating efficiency of HeLa S3 cells (3). Conditioned culture bottles were inoculated with 1 x 10^6 cells, and after 24 hr, the medium was carefully aspirated and replaced with drug-containing medium. Cell numbers were determined at 0, 24, and 48 hr in 3 to 6 bottles from each group by trypsinization. The effects of drugs on cell viability were determined by assay of colony formation as follows. Exponentially growing cells obtained from suspension cultures were plated in 15- x 60-mm plastic Petri dishes and allowed to attach for 1 hr. Drug exposures (1 and 24 hr) were started and ended by a complete change of medium. Drug-treated cultures were then incubated for 14 days, with a change of medium at 7 days, and colonies were identified by staining with crystal violet.

Neuroblastoma Cells. Mouse neuroblastoma 2A cells were obtained from the American Type Culture Collection (Rockville, MD) and were maintained as described previously (4) as monolayers in 25-cm plastic flasks in Eagle's minimal essential medium with nonessential amino acids and supplemented with 10% fetal calf serum and 2 mM HEPES. After 9 to 10 subculture generations, stock cultures were restarted from frozen cells. For experimental purposes, cells were expanded in 75-cm^2 flasks (6 x 10^5 cells/flask in 25 ml of growth medium) and were harvested after 5 to 6 days, while they were still in exponential growth (doubling time, approximately 20 hr). In subculturing stock and experimental cultures, cells were detached without trypsinization by pipetting vigorously with growth medium until a suspension of single cells was obtained (about 10 to 12 refusals). Drug effects on proliferation of neuroblastoma cells were determined as follows. Monolayer cultures were established in 25-cm^2 flasks at 2 x 10^5 cells/flask, and after 24 hr, the growth medium was carefully aspirated and replaced with drug-containing medium. Exposures of 4 hr were ended by gently rinsing monolayers with Eagle's minimal essential medium, followed by addition of drug-free growth medium. Cell numbers were determined at time zero (after removal of drug in the case of 4-hr exposures), and after 24 and 48 hr. Cell numbers were determined by gently aspirating the medium, adding a fixed quantity of 0.15 M NaCl solution back to the flasks, pipetting until a suspension of single cells was obtained, and counting with an electronic particle counter.

Uptake and Release of Vinca Alkaloids

Uptake of [3H]vinblastine and [3H]vincristine by L1210 cells was measured by a modification of a procedure described previously for use in studies of uptake of [3H]-nucleosides by suspended cells (9). Solutions containing [3H]-labeled drug at twice the concentration to be tested were prepared in Fischer's growth medium with 10% horse serum and 5 mM HEPES (pH 7.4). Uptake experiments were initiated by combining equal volumes of logarithmically growing cells (3 x 10^6 cells/ml) and the [3H]-drug solution and incubating at 37°. At graded time intervals, duplicate 1-ml samples of the reaction mixtures were transferred to 1.5-ml polypropylene microcentrifuge tubes and centrifuged 1 min in a microcentrifuge. The supernatants were aspirated, and the cell pellets were washed by resuspending in 1 ml of cold 0.15 M NaCl solution and centrifuging. The [3H] content of cell pellets was determined by removing the supernatant and solubilizing the pellet in 100 µl of 1% Triton X-100 (v/v). Tubes were uncapped, placed in plastic scintillation vials, and filled with 8 ml of a xylene:det detergent scintillant (16) for determination of radioactivity by liquid scintillation counting. In some experiments, the water content of cell pellets and the intra- and extracellular water spaces were determined by measuring uptake of [3H]sucrose or [3H]H_2O as described previously (9). The number of cells in reaction mixtures was determined by processing additional samples at each time point as described above, except that the cell pellets were resuspended in 1 ml of 0.15 M NaCl solution instead of Triton X-100, and cell concentrations were determined with an electronic particle counter.

The release of [3H]-labeled drug from cells was determined after exposing cell suspensions to drug for 4 hr at 37°. Cells exposed to [3H]-labeled drug as described above were removed from drug solutions by centrifugation and were immediately resuspended in drug-free growth medium. At timed intervals thereafter, 1-ml samples were removed and processed as above.

Chemicals

Vincristine and vinblastine were generous gifts of Eli Lilly and Co., Indianapolis, IN, and the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Radioactive drugs were purchased from Moravek Biochemicals, Brea, CA, and were repurified by high-performance liquid chromatography by a modification of a procedure described elsewhere (5). Other chemicals and plasticware were purchased from commercial sources. Cell culture reagents were purchased from Grand Island Biological Co., Burlington, Ontario, Canada.

RESULTS AND DISCUSSION

The effects of continuous exposure to vincristine and vinblastine on rates of proliferation are reported in Table 1. The IC_{50} values for the 2 drugs for a given cell line were similar, with the exception of neuroblastoma, which exhibited IC_{50} values for vincristine that were 2-fold greater than those seen for vinblastine. For each of the other 4 cell lines, the survival curves (the percentage of doublings of drug-treated cells relative to control cells over 48 or 96 hr plotted as a function of drug concentration) were basically the same for vincristine and vinblastine, indicating that, under conditions of continuous exposure, vincristine and vinblastine were equally toxic. Survival curves are presented in Chart 1 for HL-60 cells.

When the effects of short (4-hr) exposures to the 2 drugs on proliferation rates were compared, vincristine was more toxic than vinblastine against all of the cell lines except neuroblastoma (Table 2). The greatest difference was seen in the experiments

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With HL-60 cells, which were almost 40 times more sensitive to vincristine than to vinblastine (compare survival curves for 4-hr and continuous exposures in Chart 1). In contrast, although neuroblastoma cells were 2-fold more sensitive to vinblastine than to vincristine during continuous exposures, there was little difference in the effects of the 2 drugs after 4-hr exposures (Chart 2).

Differential cytotoxicity against L1210 and HeLa cells by vincristine and vinblastine was also seen when cell viability was measured by assay of colony formation (Chart 3; Table 3). For L1210 cells, vincristine and vinblastine were equally toxic after 24-hr exposures, whereas vincristine was much more toxic than vinblastine after 4-hr exposures (Chart 3). Although the IC₅₀ values obtained from the dose-response curves for 4- and 24-hr exposures to vincristine were similar, the 24-hr dose-response curve was steeper, and the apparent IC₅₀ values for drug concentrations in the experiments of Chart 3 for 4- and 24-hr exposures were 0.01 and 2 μM. A similar pattern was seen with vinblastine. The IC₅₀ values for inhibition of L1210 colony formation by vinblastine and vincristine after 4-hr exposures (Table 3) were derived from biphasic dose-response curves.⁵ These IC₅₀ values are only approximations because the plateau regions of the curves are in the area of 50% survival, and slight upward or downward shifts would cause large differences in IC₅₀ values from one experiment to the next. In all experiments except one, the vinblastine IC₅₀ was between 60 and 75 μM. HeLa cells, like L1210 cells, exhibited similar sensitivities to vincristine and vinblastine.

³ The biphasic dose-response curves suggest effects on more than one process, perhaps at different stages in the cell cycle. Studies with synchronized HeLa cells have shown that the Vinca alkaloids exhibit cytotoxic effects at multiple sites in the cell cycle.
The cloning efficiency of control cells was between 55 and 75%. A). Cells were then washed with fresh medium, diluted, and cultured in soft agar. Colony formation of L1210 cells. Exponentially growing L1210 cells (1 x 10^5 cells/ml) were exposed to vinblastine or vincristine for either 24 hr (O, Δ) or for 4 hr (■, △). Cells were then washed with fresh medium, diluted, and cultured in soft agar. For 24-hr exposures, points represent the means of one experiment each with vincristine (Δ) and vinblastine (O); bars, S.D. For the 4-hr exposures, points represent the means of 4 experiments with vincristine (A) and of one experiment with vinblastine (■), representative of 6 experiments which showed the same pattern of survival; bars, S.D. Points without bars have a S.D. less than the size of the symbol. The cloning efficiency of control cells was between 55 and 75%.

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Chart 3. Effect of 24-hr and 4-hr exposures of vinblastine and vincristine on colony formation of L1210 cells. Exponentially growing L1210 cells (1 x 10^5 cells/ml) were exposed to vinblastine or vincristine for either 24 hr (O, Δ) or for 4 hr (■, △). Cells were then washed with fresh medium, diluted, and cultured in soft agar. For 24-hr exposures, points represent the means of one experiment each with vincristine (Δ) and vinblastine (O); bars, S.D. For the 4-hr exposures, points represent the means of 4 experiments with vincristine (A) and of one experiment with vinblastine (■), representative of 6 experiments which showed the same pattern of survival; bars, S.D. Points without bars have a S.D. less than the size of the symbol. The cloning efficiency of control cells was between 55 and 75%.

Table 3

IC_{50} values for inhibition of colony formation
HeLa cells were plated in 15- x 60-mm plastic Petri dishes and allowed to grow for 24 hr, after which they were exposed to drug-containing growth medium for 1 or 24 hr. The drug-containing medium was then removed and replaced by drug-free growth medium, and cultures were incubated for 14 days before colony numbers were determined. Suspension cultures of L1210 cells (1 x 10^5/ml) were exposed to drug for 4 or 24 hr and, after one wash with fresh medium, were diluted and cultured in soft agar as described in "Materials and Methods." The IC_{50} value is the concentration of drug that reduced colony formation to 50% of control values.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Length of exposure (hr)</th>
<th>IC_{50} (nm) for Vincristine</th>
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</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>24</td>
<td>1.2 ± 0.2 (1)</td>
<td>&lt;3 ± 0.0 (1)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>33 ± 0.0 (3)</td>
<td>62 ± 0.3 (2)</td>
</tr>
<tr>
<td>L1210</td>
<td>24</td>
<td>5.4 ± 0.3 (1)</td>
<td>2.5 ± 0.0 (1)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6.0 ± 0.7 (2)</td>
<td>37 ± 0.0 (5)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of experiments used to calculate IC_{50} values.
* Mean ± S.D.

More vinblastine than vincristine was accumulated after 24-hr exposures, a result that was consistent with the effects seen when growth rates were determined during continuous exposure (Table 1). After 1-hr exposures, HeLa cells were about twice as sensitive to the cytotoxic effects of vincristine than to those of vinblastine.

The biological studies described above indicated that differential toxicity of the Vinca alkaloids was seen when cells were subjected to pulse exposures to drug, but not during continuous exposures. Experiments were undertaken with L1210 cells to determine if differences in uptake and release of the drugs by cells during and after 4-hr exposures could account for differential toxicity. Since vinblastine and vincristine are partially ionized at physiological pH (pK_{a}s are 5.0 to 5.5 and 7.4; Ref. 13), the relationship between uptake and pH was examined in the experiment presented in Chart 4. When L1210 cells were incubated for 4 hr with either drug at 50 nm, drug uptake increased as the pH increased from 5.5 to 7.5, concomitant with a loss of charge on drug molecules. Subsequent uptake experiments presented in this study were conducted at pH 7.4. The experiments of Chart 4 also indicated that L1210 cells accumulated significantly

Chart 4. Effect of pH on uptake of vincristine and vinblastine by L1210 cells. Exponentially growing cells were exposed to 50 nm [³H]vinblastine (O) or 50 nm [³H]vincristine (■) at the indicated pH. Uptake of radioactive drug (pmol/10^6 cells) was determined after 4 hr at 37° as described in "Materials and Methods." Accumulation of radioactive drug is expressed as the ratio of the apparent drug concentration within the cell (pmol drug associated with the cell pellet/aqueous volume of pellet) to the drug concentration of medium. Points, mean of duplicate determinations.

Chart 5. Uptake of vincristine and vinblastine by L1210 cells. Exponentially growing cells were exposed to [³H]vinblastine (O) or [³H]vincristine (■) at the concentrations indicated. Incubations were performed at 37° in growth medium at pH 7.4. Uptake of radioactive drug was determined after a 4-hr exposure as described in "Materials and Methods." Points, mean of duplicate determinations.

The time courses of uptake and of release of vinblastine and vincristine were investigated by measuring cellular accumulation.

The biological studies described above indicated that differential toxicity of the Vinca alkaloids was seen when cells were subjected to pulse exposures to drug, but not during continuous exposures. Experiments were undertaken with L1210 cells to determine if differences in uptake and release of the drugs by cells during and after 4-hr exposures could account for differential toxicity. Since vinblastine and vincristine are partially ionized at physiological pH (pK_{a}s are 5.0 to 5.5 and 7.4; Ref. 13), the relationship between uptake and pH was examined in the experiment presented in Chart 4. When L1210 cells were incubated for 4 hr with either drug at 50 nm, drug uptake increased as the pH increased from 5.5 to 7.5, concomitant with a loss of charge on drug molecules. Subsequent uptake experiments presented in this study were conducted at pH 7.4. The experiments of Chart 4 also indicated that L1210 cells accumulated significantly

"3H"drug (µl cell water). The latter was determined as described previously (9) from measurements of uptake of [³H]sucrose by cells in the experiment of Chart 5. Calculation of an apparent intracellular concentration assumes that cell-associated drug is unbound and is undoubtedly an overestimation.

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Differential Activity of Vinca Alkaloids

Chart 6. Time courses of uptake and release of vincristine and vinblastine by L1210 cells. Exponentially growing cells were exposed to 400 nM [3H]vincristine (A) or 200 nM [3H]vinblastine (B) either continuously for 7 hr or for 4 hr followed by culture in drug-free medium for 3 hr. For the latter, cells were collected by centrifugation and resuspended in drug-free growth medium. All incubations were performed at 37°C in growth medium at pH 7.4, and the cell content of radioactive drug was determined at timed intervals as described in "Materials and Methods."

of 3H-labeled drug at timed intervals during 4-hr exposure and by measuring loss of 3H-labeled drug at timed intervals after resuspension of cells in drug-free medium. Experiments were conducted at drug concentrations ranging from 50 to 400 nM, and time courses from a typical experiment are presented in Chart 6. Since accumulation of vinblastine at a given extracellular concentration was consistently greater than that of vincristine, uptake and release were measured under conditions in which the total cellular accumulation of each drug was the same at equilibrium. The data of Chart 6 indicate that uptake and release of vinblastine were more rapid than those of vincristine. By 15 min, when the first measurement of uptake was conducted, the cell content of vinblastine was greater than that of vincristine, and by 4 hr, when the uptake process had reached equilibrium, the cell content of both drugs was similar. When cells were resuspended in drug-free medium, the release of vinblastine was rapid, so that within 30 min, the cell content of vinblastine was significantly less than that of vincristine. Chart 7 illustrates results of 2 of a series of experiments in which the release of cell-associated drug was followed in greater detail during the first hr after cells loaded with equivalent amounts of drug were transferred to drug-free medium. The time courses for both drugs are characterized by rapid release during the first 30 min, followed by a slow, continuous release that reached an apparent equilibrium within about 3 hr. Although initial rates of release cannot be determined from the time courses of Chart 7, cells lost vinblastine more rapidly and to a greater extent than vincristine over the range of concentrations tested (50 to 200 nM vinblastine; 200 to 400 nM vincristine). These results suggest that the differential toxicity, seen when L1210 cells were exposed to vinblastine and vincristine for 4 hr, was due to differences in cellular retention of the 2 drugs.

Vincristine and vinblastine have similar constants for binding to tubulin (8.0 x 10^-6 M^-1 and 6.0 x 10^-6 M^-1, respectively) purified from other sources (15). Because L1210 cells accumulated larger quantities of vinblastine than vincristine, it seems
likely that, in addition to tubulin, vinblastine may interact with other cellular site(s) from which it readily dissociates when cells are placed in drug-free medium. One possibility is that vinblastine partitions into the lipid phase of the plasma membrane. At the controlled pH of these experiments (7.4), vinblastine has a much larger octanol:water partition coefficient (2000) than does vincristine (160) (13). Thus, significant quantities of vinblastine may partition in the membrane, thereby limiting the concentration of vinblastine in the cytosol. Membrane-associated vinblastine would quickly reequilibrate with drug-free medium when cells are washed. Although vincristine probably also partitions in the plasma membrane, during a 4-hr exposure there may be a higher concentration of free vincristine, than vinblastine, in the cytosol available for binding to tubulin.

Further evidence for association of vinblastine with the membrane was the extreme fragility of L1210 cells seen with vinblastine but not with vincristine. At higher concentrations (50 to 100 \( \mu M \)) of vinblastine, L1210 cells had to be handled carefully when washing to avoid cell lysis. Cell loss was insignificant at all concentrations of vincristine and at vinblastine concentrations less than 50 \( \mu M \).

The concentrations used in this study were well within the limits of clinically achievable concentrations for both long and short exposures. For example, the plasma levels of vincristine from time zero to "infinity" of adult patients who received an i.v. bolus of vincristine (0.45 to 1.30 mg/sq m) gave an area under the curve of drug concentration plotted against time of 22,000 \( \pm \) 15,000 nm\( \cdot \)min (17). In the experiments reported here, a concentration of 5 nm for 48-hr or 96-hr exposures corresponds to an area under the curve of drug concentration plotted against time, respectively, of 14,400 and 28,800 nm\( \cdot \)min. After a 1-hr infusion, plasma levels of vincristine in leukemic children were greater than 50 nm for up to 2 hr and remained between 5 and 50 nm for up to 4 hr (11). When adult cancer patients were given either vincristine or vinblastine as an i.v. bolus injection, the plasma levels of vincristine and vinblastine, respectively, dropped from 70 nm to 7 nm and from 150 nm to 10 nm between a few min and 4 hr after injection (12). The results presented here suggest that the mode of administration of vinblastine and vincristine is an important determinant of the relative cytotoxicity of the 2 drugs against neoplastic cells. Administration by infusion would be expected to result in similar cytotoxicity against the target cell population, whereas administration by i.v. bolus injection, because of differences in both pharmacokinetics and cellular release of drug, would be expected to result in greater cytotoxicity by vincristine than vinblastine.
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