Effect of Duration of Exposure to Verapamil on Vincristine Activity against Multidrug-resistant Human Leukemic Cell Lines


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ABSTRACT

Verapamil sensitizes multidrug-resistant cell lines to various heterocyclic anticancer drugs by inhibition of energy-dependent release of drug, presumably by interaction with membrane glycoproteins involved in drug efflux. This work assessed verapamil sensitization of human multidrug-resistant lymphocytic and myeloid leukemic cell lines (CEM/VLB100, HL-60/AR) to vincristine during exposures of short duration (4 h). When cells were transferred to drug-free medium immediately after simultaneous 4-h exposures to vincristine and verapamil, the antiproliferative activity of vincristine was not altered in CEM/VLB100 cells and was only moderately increased in HL-60/AR cells. In contrast, when cells were transferred to verapamil-containing medium, vincristine activity was greatly increased against both CEM/VLB100 and HL-60/AR cells. Verapamil enhanced accumulation and inhibited release of [3H]vincristine by CEM/VLB100 and HL-60/AR cells, indicating that the sensitization was due to an increase in cell-associated vincristine after transfer of cells to vincristine-free medium. Slot blot analysis of cellular RNA with the pMDRI probe revealed high levels of expression of the mdr1 gene in CEM/VLB100 cells but no detectable expression in HL-60/AR cells. Consistent with this finding, polypeptides (M, 170,000 to 180,000) that were recognized by a monoclonal antibody (C219) against P-glycoprotein were greatly overexpressed in CEM/VLB100 cells, but were expressed at low levels, if at all, in HL-60/AR cells. These results demonstrate the importance of duration of exposure to verapamil in reversing multidrug resistance, not only in cells that overexpress P-glycoprotein but also in cells, such as HL-60/AR, that express little, if any, P-glycoprotein.

INTRODUCTION

Multidrug resistance, a phenomenon originally identified in neoplastic cell lines and transplantable tumors, is a complex phenotype, which includes resistance to a group of chemotherapeutic agents that differ in their pharmacological mechanisms (1, 2). MDR cells often exhibit decreased drug accumulation (3) and increased expression, sometimes by virtue of gene amplification (4), of an integral membrane glycoprotein (M, 170,000 to 180,000), termed P-glycoprotein (5, 6). The gene (mdr1) encoding P-glycoprotein has been identified (7, 8), and P-glycoprotein, which exhibits sequence similarities with certain bacterial transport proteins (7–9), is thought to participate in energy-dependent extrusion of drug.

Drug resistance is a major cause of treatment failure in cancer therapy, and pharmacological approaches to overcome resistance have been examined in transplantable tumors and cell lines that exhibit the MDR phenotype. Verapamil has been shown to reverse multidrug resistance to varying degrees when administered together with the appropriate cytotoxic agent (10–15), and this reversal is commonly associated with increased accumulation of the cytotoxic agent (11, 16–18), an effect that has been attributed to verapamil-dependent inhibition of drug efflux from MDR cells (19, 10). A series of recent studies with membrane vesicles from MDR cell lines (19–22) have shown direct interaction of verapamil, and certain other modulating agents, with vinblastine-binding site(s) on P-glycoprotein of membrane vesicles of MDR cell lines.

In a study (23) undertaken to evaluate the potential use of verapamil to enhance the activity of vincristine against MDR cells for in vitro chemopurging of autologous bone marrow grafts, we observed that simultaneous treatment of drug-resistant human leukemic cell lines with verapamil and vincristine for 4 h was no more toxic against clonogenic cells than was treatment with vincristine alone, even though preliminary experiments had shown that verapamil enhanced accumulation of [3H]vincristine by the resistant cells. The current work was undertaken to investigate the importance of exposure sequence and duration in achieving the maximal synergistic action of verapamil and vincristine against cells that exhibit the MDR phenotype. The effects of various exposure protocols (simultaneous versus sequential) on proliferation rates and on accumulation and release of [3H]vincristine were examined in two MDR cell lines (HL-60/AR, 100-fold resistant to doxorubicin; CEM/VLB100, 100-fold resistant to vinblastine). Both MDR lines exhibit phenotypes characteristic of P-glycoprotein-associated multidrug resistance (18, 24–27): resistance to anthracyclines and vinca alkaloids; decreased drug accumulation due to increased efflux; and reversal of resistance by verapamil. We therefore assessed the expression of the mdr1 gene by “dot-blot” analysis of total cellular RNA using a complementary DNA probe (pMDRI) for the human mdr1 gene product (28) and by “immunoblot” analysis of membrane preparations using mAb C219 that recognizes P-glycoprotein (29). Although further investigation is required, the absence of detectable overexpression of P-glycoprotein in HL-60/AR cells suggests that these cells may possess a different mechanism of multidrug resistance, and of verapamil sensitization, than CEM/VLB100 cells.

MATERIALS AND METHODS

Cell Culture. CEM/VLB100 cells were selected from human leukemic lymphoblastic CCRF-CEM cells for resistance to vinblastine (24), and...
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HL-60/AR cells were selected from human promyelocytic leukemia HL-60 cells (30) for resistance to doxorubicin (25). The cell lines, which were routinely restarted after approximately 30 subculture generations from Mycoplasma-free stocks preserved in liquid nitrogen, were maintained in stationary suspension cultures as described previously (31). Cell numbers were determined by enumerating with an electronic particle counter (Model ZB; Coulter Electronics, Hialeah, FL), and actively proliferating cultures exhibited doubling times at 20 to 24 h.

The effects of short (4 h) and long (48 h) exposures to vincristine and/or verapamil on proliferation rates were determined by the procedure described by Ferguson and Cass (31). All experiments were conducted in triplicate. The IC50 value was determined graphically from semilogarithmic plots of relative proliferation rates as a function of drug concentration. For exposures to single agents, the relative proliferation rate was defined as the ratio of population doubling time of drug-treated cultures to that of untreated cultures, and for exposures to verapamil and vincristine, to that of cultures exposed to verapamil.

Drug Accumulation Studies. Intervals of drug uptake were initiated by combining actively proliferating cultures (3 x 10^6 cells/ml) with equal amounts of the appropriate serum-containing growth medium (prewarmed to 37°C) with 5 mM HEPES (pH 7.4) and [3H]vincristine (with or without verapamil) at twice the test concentrations. The cultures were incubated at 37°C and, at timed intervals, four 1-ml portions were removed and processed as described elsewhere (31) for determination of radioactive content using a xylene- detergent scintillant (32). In experiments that assessed the effects of verapamil on cellular retention of vincristine, 100-ml cultures were incubated for 4 h at 37°C with 0.2 nM [3H]vincristine in the presence or absence of 40 nM verapamil. The cultures were then divided into two equal portions to transfer to vincristine-free growth medium (using the same formulation as for proliferation experiments) with or without 10 nM verapamil. The cells were then collected by centrifugation (120 x g, 8 min, 22°C), washed once by centrifugation, resuspended at 10^5 cells/ml, and incubated for 24 h at 37°C. One-ml samples (4 per condition) were periodically withdrawn and processed for analysis of cellular content of radioactivity.

RNA Expression. A standard "dot-blot" method (33, 34) was used to analyze total cellular RNA for expression of the mdr1 gene using the pMDR1 probe (28), kindly provided by Dr. I. Roninson (Center for Genetics, University of Illinois Medical Center, Chicago, IL). The pT1 probe for the a-tubulin gene (35) was provided by Dr. P. Houghton (St. Jude Children's Research Hospital). Probes were labeled with [alpha-32P]dATP by a modification of the primer extension method (36).

Analysis of Reactivity with mAb C219. Plasma membrane fractions were prepared by a modification of a previously described method (37). The cell suspensions were diluted 10% (v/v) with distilled H2O and gently homogenized (25 strokes, type B Dounce system). The lysates were centrifuged (10 min, 1500 x g, 4°C), the supernatants were centrifuged (15 min, 30,000 x g, 4°C), and the resulting pellets were resuspended in Tris-sucrose buffer (0.5 M sucrose-20 mM Tris-HCl pH 7.4 ethylene glycol bis[b-aminoethyl ether]-N,N,N',N'-tetraacetic acid, pH 7.4) and homogenized (25 strokes, type B Dounce system). The homogenates were layered onto Percoll (17.5% in Tris-sucrose buffer, and the plasma membrane fractions were collected by centrifugation (15 min, 10,000 x g, 0.4°C) and stored in liquid nitrogen.

For SDS-PAGE, the membrane preparations were solubilized in SDS sample buffer (without boiling) and analyzed in 7.5% acrylamide gels (38, 39). Prestained protein molecular weight standards (M, 14,300 to 200,000) were run in an adjacent lane, and proteins were localized on gels by silver staining (40). Polypeptides were electrophoretically transferred (35 V, 16 h) from the polyacrylamide gel onto polyvinylidene difluoride transfer membranes (41). P-glycoprotein was identified by reactivity with mAb C219, using an alkaline phosphatase detection system and the method of Blake et al. (42), modified by the substitution of 0.1 nM Tris (pH 9.3) for veronal acetate.

Materials. [3H]Vincristine (10 Ci/mmole) was purchased from Moravek Biochemicals (Brea, CA) and was repurified within 4 days of use by high-pressure liquid chromatography using a reverse-phase Partisil 10/25 ODS-3 column (Whatman Chemical Separations, Clifton, NJ) and a gradient of 45 to 80% methanol in 10 mM KH2PO4 (pH 4.9) over 40 min (31). Specialized reagents were obtained from the following companies: vincristine sulfate (Eli Lilly and Co., Indianapolis, IN); verapamil (Sigma Chemical Company, St. Louis, MO); polyvinylidene difluoride transfer membranes (Millipore, Ltd., Mississauga, ON); mAb C219 (Centocor, Malvern, PA); alkaline phosphatase conjugates of goat anti-mouse IgG (heavy and light) and electrophoresis reagents (Bio-Rad, Mississauga, ON); [alpha-32P]dATP (Dupont/New England Nuclear, Boston, MA); and tissue culture materials (Grand Island Biological Co., Burlington, ON). Other reagents were purchased from standard commercial sources.

RESULTS

Growth-inhibitory Effects of Verapamil against Drug-sensitive and Drug-resistant Cells. Long-term exposures to verapamil inhibited proliferation of the parent and MDR lines, and, although not rigorously determined, IC50 values for verapamil inhibition of proliferation rates were >40 nM since 48-h exposures to 40 nM verapamil inhibited proliferation rates of HL-60 and HL-60/AR cells by 40 to 50% and of CCRF-CEM and CEM/VLB100 cells by 30 to 40% (data not shown). The IC50 value for CCRF-CEM cells has previously been reported to be 40 nM (13). In contrast, 4-h exposures to graded concentrations of verapamil (≤40 nM) were without effect on proliferation rates of either the parent or MDR cells (data not shown). In the studies reported below, verapamil exposures of 4 h were limited to 40 nM, and exposures of 48 h were limited to either 5 or 10 nM verapamil since the objective of the study was to examine the ability of nontoxic exposures of verapamil to sensitize MDR cells to vincristine.

Effects of Verapamil on Vincristine Resistance during Continuous Exposures. The MDR cell lines used in this study were originally selected for resistance to vinblastine (CEM/VLB100) and to doxorubicin (HL-60/AR) and were found to be cross-resistant, although to a lesser degree, to other classes of natural product drugs (Refs. 24 to 26; Footnote 5). As was shown previously (25, 27), CEM/VLB100 cells were strongly resistant to vincristine, whereas HL-60/AR cells were much less so (Table 1). Verapamil enhanced the antiproliferative activity of vincristine in both MDR lines, reducing the relative resistance of CEM/VLB100 cells from 967- to 17-fold and that of HL-60/AR cells from 17- to 1-fold.

Effects of Verapamil on Vincristine Resistance during 4-h Exposures. The experiments of Table 2 were undertaken to determine if the presence of verapamil in culture fluids after termination of vincristine exposures enhanced the biological activity of vincristine after short exposures. Cells were incubated for 4 h in growth medium containing graded concentrations of vincristine with or without verapamil, followed by an additional 48 h in vincristine-free medium with or without verapamil for determination of proliferation rates.

In the absence of verapamil, substantially higher concentrations of vincristine were required to inhibit proliferation rates after 4-h exposures than after 48-h exposures. The IC50 values for 4-h exposures to vincristine differed from those obtained during 48-h exposures by 12- and 19-fold, respectively, for HL-60 and CCRF-CEM cells and by 232- and 268-fold, respectively, to vincristine.4

4 In a separate study (23) that assessed survival of clonogenic cells (HL-60, HL-60/AR, CCRF-CEM, CEM/VLB100), 4-h exposures to 40 nM verapamil had no effect on colony formation, whereas 24-h exposures decreased colony formation by 41, 24, 10, and 30%, respectively.

CEM/VLB100 cells exhibited levels of resistance of 269, 1163, and 109, respectively, to vinblastine, vincristine, and doxorubicin (24, 26); HL-60/AR cells exhibited levels of resistance of 1111, 2.25, and 7.5, respectively, to doxorubicin, vinblastine, and vincristine. (25).

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Table 1 Effects of verapamil on vincristine resistance during prolonged exposure to both drugs

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC (_{50}) (µM)</th>
<th>IC (_{50}) (µM)</th>
<th>Fold resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Verapamil + Verapamil</td>
<td>Verapamil - Verapamil</td>
<td></td>
</tr>
<tr>
<td>HL-60</td>
<td>0.004 ± 0.002* 0.002 ± 0.001</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>HL-60/AR</td>
<td>0.059 ± 0.012 0.004 ± 0.001</td>
<td>17.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>0.001 ± 0.000 0.001 ± 0.000</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CEM/VLB(_{100})</td>
<td>0.967 ± 0.021 0.017 ± 0.004</td>
<td>967.0</td>
<td>17.0</td>
</tr>
</tbody>
</table>

* Mean ± SD of 3 or 4 experiments.

Table 2 Effects of verapamil on resistance to 4-h exposures to vincristine: simultaneous versus sequential administration

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC (_{50}) (µM)</th>
<th>IC (_{50}) (µM)</th>
<th>Fold resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-60</td>
<td>0.035 ± 0.007 0.007 (5')</td>
<td>0.002 (18) 0.004 (9)</td>
<td></td>
</tr>
<tr>
<td>HL-60/AR</td>
<td>16.3 ± 5.7 0.340 (48)</td>
<td>0.215 (76) 0.025 (652)</td>
<td></td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>0.019 ± 0.7 0.015 (1)</td>
<td>0.015 (1) 0.007 (3)</td>
<td></td>
</tr>
<tr>
<td>CEM/VLB(_{100})</td>
<td>264.0 ± 26.2 251.0 (1)</td>
<td>15.5 (17) 0.235 (1233)</td>
<td></td>
</tr>
</tbody>
</table>

* When cells were subjected to 4-h exposures to 40 µM verapamil alone, proliferation rates were inhibited by <5%. When cells were subjected to the various manipulations that involved 48-h exposures to verapamil, proliferation rates were inhibited by 10 to 15%.

* Mean ± SD given for untreated cultures.

Numbers in parentheses (fold-decrease), ratio of IC\(_{50}\) value of untreated culture to that of the corresponding verapamil-treated culture.

Fig. 1. Effects of simultaneous and sequential exposures to verapamil on accumulation and release of vincristine by CCRF-CEM and CEM/VLB\(_{100}\) cells. Cultures were established and incubated for 4 h in growth medium containing 0.2 µM [\(^3\)H]vincristine alone (○, □) or with 40 µM verapamil (△, △), after which the cells were washed and further incubated in vincristine-free medium without verapamil (○, △) or with 10 µM verapamil (□, □). Vincristine accumulation was calculated from measurements of cell-associated radioactivity (2 determinations per condition) and cell concentrations (2 determinations per condition). The designations used to indicate the duration of verapamil exposures are: —/—, no verapamil; +/-, 40 µM verapamil during accumulation; —/+ , 10 µM verapamil during release; +/+, 40 µM verapamil during accumulation and 10 µM verapamil during release. Each panel represents the results of a single representative experiment.

AR cells during the release phase at 10, rather than 5, µM.

The results demonstrated greater accumulation of vincristine by the parent lines than by the corresponding MDR lines in the absence of verapamil. Enhanced accumulation of vincristine in the presence of 40 µM verapamil was evident with CCRF-CEM cells (Fig. 1, top), but not with HL-60 cells (Fig. 2, top), although enhancement was seen with HL-60 cells at other vincristine concentrations (see below). The kinetics of vincristine accumulation differed in that there was a gradual increase of cell-associated vincristine by CCRF-CEM cells, whereas maximal accumulation by HL-60 cells was reached within 30 min. For both HL-60 and CCRF-CEM cells, vincristine release was inhibited by the presence of 10 µM verapamil in culture fluids, and the effects of verapamil on cell-associated vincristine were evident as late as 24 h after transfer of cells to vincristine-free medium.

Coadministration of 40 µM verapamil with 0.2 µM vincristine resulted in major increases in cell-associated vincristine in the MDR lines. At the end of 4-h exposures, the levels of vincristine were 5-fold greater in verapamil-treated, than in untreated, CEM/VLB\(_{100}\) cells (Fig. 1, bottom) and 2-fold greater in verapamil-treated HL-60/
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Fig. 2. Effects of simultaneous and sequential exposures to verapamil on accumulation and release of vincristine by HL-60 and HL-60/AR cells. The experimental manipulations were exactly as described in Fig. 1. Cultures were incubated for 4 h in growth media containing 0.2 μM [3H]vincristine alone (○, ●) or with 40 μM verapamil (□, ▲), after which the cells were washed and further incubated in vincristine-free media without verapamil (●, ▲) or with 10 μM verapamil (□, △). The designations used to indicate the duration of verapamil exposures are: —/+, no verapamil; +/—, 10 μM verapamil during accumulation; —/+, 40 μM verapamil during accumulation and 10 μM verapamil during release. Each panel represents the results of a single representative experiment.

Table 3 Effects of simultaneous and sequential administration of verapamil on vincristine exposures (concentration × time)

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Effective drug exposure (pmol × h/10^6 cells) at the following durations of verapamil exposures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4'×/4'</td>
</tr>
<tr>
<td>HL-60</td>
<td>18.4</td>
</tr>
<tr>
<td>HL-60/AR</td>
<td>7.6</td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>78.9</td>
</tr>
<tr>
<td>CEM/VLBh10</td>
<td>4.7</td>
</tr>
</tbody>
</table>

* Numbers in parentheses (fold-increase) ratio of the effective drug exposure of verapamil-treated culture to that of the corresponding untreated culture.

The areas under the curves (Figs. 1 and 2) for cellular drug content versus time (from 0 to 8 h) were calculated to obtain a measure of the effective drug exposure during the accumulation phase and the first 4 h of the release phase.

Table 3 illustrates the effects of simultaneous and sequential administration of verapamil with vincristine on effective drug exposures during the first 8 h of the experiments of Figs. 1 and 2. Verapamil increased effective drug exposures, both when coadministered with vincristine and when administered after termination of vincristine exposures. The cells that were subjected to both simultaneous and sequential treatment with verapamil experienced the greatest effective drug exposure to vincristine, suggesting that maximum sensitization of MDR cells can be achieved by administration of verapamil both during and after short exposures to vincristine.

Effects of Verapamil on Vincristine Accumulation by Drug-sensitive HL-60 Cells during 4-h Exposures. Although verapamil enhanced the antiproliferative activity of vincristine against HL-60 cells, the results of the uptake experiments of Fig. 2 were inconclusive with respect to stimulation of vincristine accumulation by verapamil. In dose-response studies that were undertaken to assess the extent to which verapamil altered accumulation of vincristine (Fig. 3), HL-60 cells were incubated for 4 h with [3H]vincristine in the presence or absence of verapamil in growth medium under conditions similar to those of the proliferation experiments. When cells were exposed simultaneously to a growth-inhibitory concentration of vincristine (0.1 μM) and increasing concentrations of verapamil (0 to 50 μM), cell-associated vincristine was increased (Fig. 3A), and the increase was proportional to concentration over the range of verapamil concentrations that were tested. The presence of 40 μM verapamil enhanced accumulation over a broad range of vincristine concentrations (Fig. 3B), and, in both the presence and absence of verapamil, the relations between cell-associated
vincristine and concentration were linear.

Analysis of Cells for Expression of P-Glycoprotein. Overexpression of P-glycoprotein is considered the hallmark of the “classic” MDR phenotype, and cells of the CEM/VLB100 line exhibit high levels of P-glycoprotein, as judged by reactivity of RNA with mdr1 probes (27) and of membrane proteins with mAb C219 (29). In the experiments of Fig. 4, the parent and MDR lines were examined for expression of the mdr1 gene using the pMDR1 probe, which contains segments of the human mdr1 gene (28). The pMDR1 probe hybridized with cellular RNA from CEM/VLB100 cells but not with RNA from CCRF-CEM cells, indicating considerable overexpression of mdr1 mRNA in CEM/VLB100 cells. There was no detectable expression of the mdr1 gene in HL-60 or HL-60/AR cells.

In the experiments of Fig. 5, plasma membranes from both pairs of cell lines were examined for expression of P-glycoprotein by “immunoblot” analysis with mAb C219, which recognizes an epitope conserved in human cells (29). The immunoblot assay was evidently more sensitive than the dot blot assay, since immunoreactive material (M, 180,000) was detected in preparations from CCRF-CEM cells, which were negative in the dot blot assay of Fig. 4. Overexpression of immunoreactive material in CEM/VLB100 cells was clearly evident. Although immunoreactive material of the same mobility (M, 180,000) was also detected in the membrane preparations from HL-60 and HL-60/AR cells, the bands were very faint, and the quantity of immunoreactive material appeared to be less than that found in drug-sensitive CCRF-CEM cells. It was not possible to determine if HL-60 and HL-60/AR cells contained different quantities of immunoreactive material.

DISCUSSION

In this work, we have examined the effects of long (48 to 72 h)- and short-term (4 h) exposures to vincristine and verapamil on two MDR human leukemic cell lines that were obtained by selection with different natural product drugs (24–26). When resistance was assessed by determining IC50 values for inhibition of proliferation rates during long-term exposures to vincristine, the absence of verapamil, CEM/VLB100 and HL-60/AR cells were, respectively, about 1000- and 20-fold resistant to vincristine, relative to the corresponding drug-sensitive cells. However, when resistance was assessed during short-term exposures, CEM/VLB100 and HL-60/AR cells were, respectively, about 14,000- and 500-fold resistant to vincristine. Thus, the degree of resistance in both MDR lines is greater for vincristine exposures of short, than of long, duration.

Since the demonstration by Tsuruo et al. (10) of reversal of the MDR phenotype in the P388 leukemia by coadministration of verapamil with vinblastine, there have been numerous reports of synergism between verapamil and the Vinca alkaloids. Most workers have monitored proliferation rates during long exposures, usually 48 or 72 h, and there have been only a few studies (43, 44) in which clinically more relevant exposures (45) of short duration have been evaluated. The results presented here demonstrate that the ability of verapamil to reverse resistance, when coadministered with vincristine, is diminished when drug exposures are shortened, particularly in cells with high levels of resistance to vincristine. For CEM/VLB100 cells, coadministration of vincristine and verapamil during 48-h exposures reduced resistance from 967-fold to 17-fold, whereas there was no effect on resistance during 4-h exposures. For HL-60/AR cells, coadministration of verapamil with vincristine during 48-h exposures completely reversed resistance, whereas only partial reversal was achieved during 4-h exposures. A greater reversal of resistance was seen in both MDR lines when cells were exposed to verapamil after 4-h exposures to vincristine than when cells were exposed for 4 h to both drugs together. The greatest enhancement of vincristine activity was seen when verapamil was present in culture medium both during and after the 4-h vincristine exposures.

Coadministration of verapamil with the various drugs associated with the MDR phenotype often results in increased net

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In humans, after i.v. bolus injections of vincristine, >99% of the total dose is cleared from the serum within 4 h (45).

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Fig. 4. Analysis of drug-sensitive and -resistant cells for expression of the mdr1 gene. RNA was extracted from cells, denatured and “dot-blotted” onto nitrocellulose for analysis of reactivity with the pMDR1 probe and, to control for RNA loading, the pT1 probe for α-tubulin. The number of cells used for preparation of each extract is indicated.

Fig. 5. Analysis of plasma membrane preparations of drug-sensitive and -resistant cells for the presence of P-glycoprotein. Plasma membrane fractions from HL-60/AR cells (lane 1), HL-60 cells (lane 2), CEM/VLB100 cells (lane 3), and CCRF-CEM cells (lane 4) were subjected to SDS-polyacrylamide gel electrophoresis (8 μg per lane) and proteins were electrophoretically transferred for “immunoblot” analysis with mAb C219. Arrow, position of P-glycoprotein.

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5' mdr  pT1

Cells x 10^-5 / dot

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accumulation of drug in MDR cell lines (11, 16–18, 25), and these effects have been previously demonstrated in both CEM/ VLB100 and HL-60/AR cells (18, 25, 26). Although the molecular mechanism remains to be determined, inhibition of drug release, rather than drug uptake, has been shown to be the basis of the increased cellular accumulation of drug seen in the presence of verapamil (10, 16, 25). In both MDR lines used in this work, the cellular content of [3H]vincristine was increased when verapamil and vincristine were coadministered during the 4-h accumulation phase. In addition, the loss of [3H]vincristine presence of verapamil (10, 16, 25). In both MDR lines used in
ular mechanism remains to be determined, inhibition of drug
role of P-glycoprotein in mediation of these effects is not
be explained by inhibition of vincristine release by verapamil.
Verapamil also inhibited release of vincristine from the parent
cells, indicating that its actions are not limited to MDR cells.
The role of P-glycoprotein in drug resistance (47).

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