Sensitive and Rapid Bioassay for Analysis of P-Glycoprotein-inhibiting Activity of Chemosensitizers in Patient Serum

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ABSTRACT

Clinical studies of agents capable of reversing P-glycoprotein (Pgp)-mediated multidrug resistance have attracted much attention in recent years. One question of interest in such studies is whether the concentrations achieved by chemosensitizers are sufficient to inhibit Pgp function. The goal of the present study was to develop a reliable ex vivo bioassay for analysis of the Pgp-inhibiting activity of chemosensitizer-containing patient serum. The fluorescent Pgp substrates daunorubicin (DNR) and rhodamine 123 (R123) were used as probes for Pgp function. The 8226/DOX6 human myeloma cell line, which expresses Pgp at levels that can be detected in clinical cancers, was used as a model system. The index chemosensitizers tested were dexverapamil (DVPM) and cyclosporin A, with particular focus on DVPM. Using flow cytometry, chemosensitizer effects on 1-h drug accumulation and on drug retention at 30 min were evaluated. In the studies using pooled human serum spiked in vitro with graded chemosensitizer concentrations, the order of assay sensitivity was R123 retention >> DNR retention >> DVPM retention. Keeping serum spiked with DVPM for several hours at room temperature or 4°C or for several months at -80°C had no effect on Pgp-blocking activity. Sixteen blood samples from patients with metastatic breast cancer receiving DVPM to overcome epirubicin resistance were analyzed for Pgp-inhibiting activity and for levels of DVPM and nor-DVPM, the major metabolite of verapamil. Each patient sample was found capable of increasing R123 retention in the 8226/DOX6 cells, with activity factors of 3- to 8-fold and good agreement between DVPM blood levels and bio assay activity (r = 0.7168; two-sided P = 0.0018). The R123 retention assay developed and validated in this study seems to be a sensitive, reproducible, and easy-to-use method for analysis of Pgp-inhibiting activity of chemosensitizer-containing human serum. The assay seems capable of estimating DVPM blood levels and could prove to be a valuable tool for monitoring chemosensitizer treatment in cancer patients.

INTRODUCTION

The concept of reversing Pgp-associated MDR has been attracting much attention in recent years (1-3). A number of clinical studies of so-called chemosensitizers, agents that are able to overcome Pgp-associated MDR, have been conducted (4). In some hematological malignancies, i.e., multiple myeloma and malignant lymphomas, racemic verapamil, CSA, and, most recently, DVPM have been found capable of reversing clinical chemotherapy resistance (5-8). In the many studies conducted in solid tumors, however, the addition of chemosensitizers to chemotherapy usually has failed to overcome resistance. One question raised by these data has been whether the blood levels achieved by chemosensitizers in patients are adequate to inhibit Pgp function. In some clinical studies, attempts to analyze the ability of chemosensitizer-containing patient serum to reverse MDR in vitro have been reported using a variety of approaches, including growth inhibition and drug accumulation assays (9-15). However, the data from these studies seem inconclusive, because only a few clinical samples were usually analyzed, little or no data have been reported regarding assay sensitivity and specificity, and the applied approaches have not been validated.

The present work represents an attempt to develop a sensitive, specific, and rapid bioassay for ex vivo analysis of the Pgp-inhibiting activity of chemosensitizer-containing patient serum. Studies were carried out with CSA and DVPM, chemosensitizers representing the two classes of agents that seem most promising for clinical use at the present time. The particular focus of this work was on DVPM, because a clinical Phase II study was concurrently in progress evaluating oral DVPM for overcoming epirubicin resistance in patients with metastatic breast cancer. Accordingly, we had the opportunity to validate the experimental bioassay data in clinical material.

MATERIALS AND METHODS

Cell Lines. The cell line model used in these studies was the 8226/DOX6 human myeloma subline, which exhibits all characteristics of the Pgp-associated MDR phenotype (16). The 8226/DOX6 cells have been shown previously to express Pgp at levels similar to myeloma cells from patients with drug-refractory disease (17). The parental RPMI 8226 myeloma cell line

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The abbreviations used are: Pgp, P-glycoprotein; MDR, multidrug resistance; R123, rhodamine 123; DNR, daunorubicin; DVPM, dexverapamil; CSA, cyclosporin A.

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was used as a Pgp-negative control where indicated. Both cell lines were kind gifts from Dr. W. S. Dalton (The University of Arizona Cancer Center, Tucson, AZ). The parental cell line was obtained originally from the American Type Culture Collection (Rockville, MD). Cells were grown in suspension culture using RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% (v/v) penicillin/streptomycin (all from Gibco BRL, Life Technologies AG, Basel, Switzerland), and 1% (v/v) L-glutamine (Inotech AG, Dottikon, Switzerland) at 37°C in humidified air with 6% CO₂. Prior to use in experiments, the 8226/DOX6 cells were grown in the absence of doxorubicin for a minimum of 1 week. All experiments were carried out using exponentially growing cells. Using Hoechst dye 33258, cell lines were checked regularly for Mycoplasma infection (18) and found to be negative.

**Drugs and Reagents.** Human serum was purchased from Inotech and was proven by biochemical analysis to contain normal amounts of total protein (76 g/liter), albumin (55 g/liter), α₁-globulin (7.7 g/liter), and α₁-acid glycoprotein (0.85 g/liter). R123 and DNR were used as fluorescent Pgp substrates. R123 was purchased from Sigma (Buchs, Switzerland), and DNR was from Pharmacia Farmitalia (Zug, Switzerland). DVPVM and nor-DVPVM were kind gifts from Knoll AG (Ludwigshafen, Germany). The source of CSA used in these studies was Sandimmun IV (Sandoz, Basel, Switzerland), which contains Cremophor EL as a vehicle for the water-insoluble cyclosporin. Cremophor EL has been shown previously to be capable of reversing Pgp-associated MDR in preclinical models (19). DVPVM, nor-DVPVM, R123, and DNR were dissolved in double-distilled water. Stock concentrations of R123 and DNR were prepared by using RPMI 1640 medium. Separate stock concentrations of the chemosensitizers were prepared for experiments carried out in medium and in human serum by using the respective materials.

**Drug Accumulation and Retention Studies.** Cellular accumulation and retention of DNR or R123 were analyzed by flow cytometry (FACScan; Becton Dickinson, San Jose, CA). Preliminary experiments indicated that the fluorescence spectra of the chemosensitizers did not interfere with the fluorescence emitted by either DNR or R123. Studies were done in medium supplemented with 1% (v/v) L-glutamine and 2% fetal bovine serum (pharma medium) and in 100% human serum. In the accumulation studies done in pharma medium and in human serum, respectively, cells were incubated with 1.5 and 3.0 μg/ml DNR. The respective concentrations used of R123 were 0.1 and 0.5 μg/ml. These concentrations of the two Pgp fluorophores were demonstrated in preliminary experiments to be optimal for the evaluation of chemosensitizer effects under the particular experimental conditions. One million cells/ml were incubated at 37°C with either of the Pgp probes in the absence or presence of the chemosensitizer. At 1 h, cells were washed twice with ice-cold PBS, resuspended in ice-cold PBS, and kept on ice until analysis of cellular DNR or R123 fluorescence were 585 and 530 nm, respectively.

In drug retention studies, 1 × 10⁶/ml cells were loaded with either 3.0 μM DNR or 1 μg/ml R123 for 1 h at 37°C in pharma medium and then washed twice with ice-cold PBS. For analysis of baseline drug accumulation, aliquots of cells were processed further as described above for drug accumulation studies. The cells used to evaluate chemosensitizer effects on drug retention were resuspended in 0.5 ml 37°C pharma medium or pooled serum with or without the chemosensitizer or in patient serum and reincubated at 37°C. At 30 min, cells were washed twice with ice-cold PBS, resuspended in ice-cold PBS, and kept on ice until analysis of the cellular daunorubicin or R123 content.

In the experimental studies and in the controls of clinical samples, serum was spiked with the chemosensitizer by adding the particular concentrations immediately prior to use. All analyses were performed in triplicate. In both accumulation and retention assays, chemosensitizer activity was defined as fold increase in cellular fluorescence intensity, calculated as the ratio of fluorescence values obtained with and without the chemosensitizer. Activity factors of clinical samples were calculated by comparing the cellular content of the index agent when using patient serum versus pooled human serum without the chemosensitizer.

**Patient Samples.** For analyses of DVPVM and nor-DVPVM plasma levels and bioassay activity, 16 blood samples were obtained from patients with metastatic breast cancer who participated in a multi-institutional Phase II study of DVPVM in combination with epirubicin. The study, including the collateral laboratory studies, was approved by the Ethical Committee of the Kantonsspital St. Gallen, and written informed consent was obtained from the patients. One eligibility criterion for study entry was that patients did not take other drugs known to reverse Pgp-associated MDR. DVPVM (Knoll AG) was given p.o. at a dose of 300 mg every 6 h for a total of 13 doses. Epirubicin was administered on day 3, 6 h after the ninth DVPVM dose. Blood samples for *in vitro* analyses were drawn immediately prior to the start of DVPVM administration and epirubicin infusion. For bioassay studies and measurements of trough levels of DVPVM and nor-DVPVM, serum and plasma were obtained and stored at −80°C until analysis.

Bioassay analyses of sets of clinical samples were performed on various days. Because the studies of DVPVM-spiked serum showed some day-to-day variation in DVPVM activities, the activity factors produced by the clinical samples were normalized for analyses of correlations, e.g., between plasma levels and activity. Theoretical activity factors were calculated for the particular DVPVM concentrations according to: (a) the dose-effect curve derived from the data base of all 17 experiments with DVPVM-spiked serum; and (b) the dose-effect curve derived from the particular DVPVM-spiked serum controls coprocessed with the clinical samples. Dividing theoretical activity *a* by *b* resulted in the normalization coefficient, which was used to correct the measured patient sample activity.

**Quantitation of DVPVM Plasma Levels.** Plasma concentrations of DVPVM and nor-DVPVM were analyzed by using a previously described specific and highly sensitive high-performance liquid chromatographic method (20) with slight modifications. Briefly, DVPVM, nor-DVPVM, and the internal standard D-517 were extracted from alkalized plasma with n-heptan/isopropanol and re-extracted into dilute sulfuric acid. Separation was performed by reverse-phase liquid chromatography (Li-chrospher 60; reverse-phase select B, 5 μm) with a mobile phase consisting of a mixture of 0.05 M KH₂PO₄, acetonitrile, and...
correlation, as applicable, was used to analyze statistical agreement between data sets. Data were considered statistically significant at two-tailed P < 0.05. All statistical analyses were carried out by using the InStat program (Graph Pad, San Diego, CA) for the Macintosh (Apple Computer Co., Cupertino, CA).

RESULTS

First, we evaluated the effects of human serum spiked with graded concentrations of DVPM or CSA when using DNR or R123 as Pgp probes and drug accumulation or retention as assay end points. In this series of studies, retention of R123 was identified as the best assay end point with respect to the absolute levels of activity achieved by the chemosensitizers and the steepness of the dose-effect curve, i.e., the ability of an assay to discriminate between the effects of small increments in dose (Fig. 1). Another distinct advantage of using R123 rather than DNR was that over the retention period of 30 min, no R123 was lost by the parent cell line, whereas >90% was effluxed by the 8226/DOX6 cells. The respective figures for DNR were ~30% and ~70%, respectively. The percentage of R123 lost by the Pgp-positive cell line was the same when incubated in pharma medium or in human serum. Based on these data, the R123 retention assay was considered the method of choice for analysis of clinical samples; therefore, all further analyses were carried out with this particular assay.

Clinical serum samples can be subjected to various conditions that may affect laboratory analyses, e.g., standing at room temperature or 4°C for hours or storage at −80°C for months. When DVPM-spiked serum was subjected to these particular conditions prior to analysis, no effect on bioassay activity was observed (data not shown).

When analyzing patient samples, pooled human serum without the chemosensitizer and containing 0.5, 1, 2, 4, and 6 μM DVPM were coprocessed as controls. These particular DVPM concentrations were selected because experimental modeling showed them to yield a dose-effect curve that was superimposable on the curve derived from 24 DVPM dose increments of 0.25 μM each (Fig. 2). In the initial analysis of the bioassay data from the clinical samples, we assumed nor-DVPM and DVPm to be equipotent in increasing R123 retention. Accordingly, the sum of DVPM and nor-DVPM concentrations measured in the patient samples was used as the basis for calculating theoretical activities. However, poor agreement was found between measured activities and the theoretical activities derived for particular concentrations from the DVPM control curves (data not shown). This prompted us to evaluate the effects of nor-DVPM versus DVPM on the cellular pharmacology of R123 in 8226/DOX6 cells. In these studies, nor-DVPM was found to be clearly more potent than the parent compound in increasing R123 accumulation, in both pharma medium and pooled human serum (Fig. 3). Importantly, the same was true in the R123 retention assay (Fig. 4), with 2.3–3.1-fold higher

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**Fig. 1** Comparative activities of DVPM-spiked human serum on accumulation and retention of R123 and DNR in 8226/DOX6 cells. Points, mean; bars, SE of at least three independent experiments, each performed in triplicate. Where bars are not shown, SEs are included in symbols.

**Fig. 2** Dose-effect curves for DVPM in the R123 retention assay. Pooled human serum was spiked with 0.25–6 μM DVPM at dose increments of 0.25 μM, and activity on R123 retention in 8226/DOX6 cells was evaluated in five independent experiments. Two dose-effect curves were generated, based on all 24 concentrations and on the index concentrations of 0.5, 1, 2, 4, and 6 μM. The two curves are completely superimposable and, thus, cannot be distinguished in this plot. Points, mean; bars, SE of five independent experiments, each performed in triplicate. Where bars are not shown, SEs are included in symbols.
activity of nor-DVPM at the concentrations measured in the clinical samples. Accordingly, the data from the clinical samples were reanalyzed by calculating the theoretical activities of DVPM and nor-DVPM separately to arrive at the total theoretical activity of the particular samples. The dose-effect curve used for calculation of the theoretical nor-DVPM activity was derived from the data base of 7 experiments carried out with pooled serum spiked with 0.5, 1, 2, 4, and 6 µM nor-DVPM (Fig. 4). DVPM and nor-DVPM plasma levels measured in the 16 clinical samples and corresponding bioassay activities are listed in Table 1. Significant agreement was found between total theoretical activities of patient samples and normalized measured activities (r = 0.553; P = 0.0132) and between combined DVPM plus nor-DVPM plasma levels and normalized bioassay activities (Fig. 5). The correlation between activity factors and total DVPM levels remained significant (r = 0.66; P = 0.005) when the measured nor-DVPM levels were converted to the corresponding equipotent DVPM concentrations (plot not shown). None of the clinical samples taken prior to the start of DVPM administration showed an effect on R123 retention when compared with the pooled human serum controls. From six clinical patient samples containing DVPM, sufficient serum was available to analyze additionally effects on DNR and R123 accumulation and DNR retention. The activity factors measured for DNR accumulation ranged from 1.03–1.25-fold, for R123 accumulation and DNR retention. The activity factors measured for DNR accumulation ranged from 1.12–1.57-fold, and for DNR retention from 1.09–1.69-fold, respectively (data not shown). None of the samples had an effect on drug accumulation in the parental cell line.

**DISCUSSION**

In clinical studies of chemosensitizers, blood levels are targeted usually, which, based on experimental data, are believed to be needed for effective reversal of Pgp-associated MDR. In fact, however, little is known about the ability of these chemosensitizer concentrations to inhibit Pgp function when present in man. Many chemosensitizers, for example, are bound extensively by serum proteins, and *in vitro* testing in the presence of physiological levels of drug-binding proteins has been found to diminish significantly the effectiveness of some agents in reversing Pgp-associated MDR (21–24). Hence, direct analysis of chemosensitizer-containing patient samples seems needed to assess their ability to inhibit Pgp function.

One approach to achieve this goal is the use of *ex vivo* bioassays. To be useful, such assays must be sensitive and reproducible. Ideally, they should be rapid, inexpensive, and able to be performed readily by routine laboratories. The R123 retention assay developed in the present studies seems to meet these criteria. The assay was found to be highly sensitive with respect to two clinically important requirements, *i.e.*, the ability to measure effects produced by low chemosensitizer concentrations and the ability to discriminate between effects of small dose increments. The basis for the assay’s sensitivity seems to be the particularly efficient Pgp-mediated efflux of R123. Within 30 min, the 8226/DOX6 cells effluxed >90% of R123, whereas essentially no R123 was lost by the parental cell line. Points, mean; bars, SE of at least three independent experiments, each performed in triplicate. Where bars are not shown, SEs are included in symbols.

*Fig. 3* Comparative effects of DVPM and nor-DVPM on R123 accumulation in 8226/DOX6 cells. Experiments were carried out in pharma medium (PM) and pooled human serum (HS) using R123 at 0.1 and 0.5 µg/ml, respectively. The average reduction in basal accumulation of R123 in 8226/DOX6 versus parental cells was 11-fold. Points, mean; bars, SE of at least six independent experiments, each performed in triplicate. Where bars are not shown, SEs are included in symbols.

*Fig. 4* Effects of DVPM, nor-DVPM, and CSA on R123 retention in 8226/DOX6 cells when tested in human serum. Cells were loaded in pharma medium with R123, washed with ice-cold PBS, and resuspended in pooled human serum in the absence or presence of the chemosensitizer. After 30 min incubation at 37°C, cells were washed with ice-cold PBS, and the amount of R123 retained was analyzed by flow cytometry. In the absence of the chemosensitizer, the average decrease in R123 content in 8226/DOX6 was 95% or 20-fold, whereas no R123 was lost by the parental cell line. Points, mean; bars, SE of at least three independent experiments, each performed in triplicate. Where bars are not shown, SEs are included in symbols.
DVPM concentrations in patient samples and their bioassay activities and, particularly, between drug levels and Pgp-inhibiting activity. Due to the lack of appropriate clinical material, we were not able to validate the R123 retention assay for cyclosporins. However, in the studies using spiked serum, assay sensitivity and reproducibility were found to be quite similar when testing DVPM or CSA. Studies of the CSA derivative PSC 833, which is being used increasingly in clinical trials, were not performed. The differences in results observed between clinical and spiked DVPM samples are to be expected in this type of comparative study. One important variable that, for instance, cannot be controlled in experimental samples is that cancer patients may take chemical compounds that we do not know about and that may affect Pgp function in their own right or may interfere with protein binding of the chemosensitizer. In this study, the baseline control samples taken prior to the start of DVPM administration were not found to increase R123 retention. Another factor could be the concentrations of drug-binding serum proteins, which can vary in tumor patients. This, in turn, may result in differences in the levels of non-protein-bound drug and, thus, Pgp-inhibiting activity. The patients in this study were found to have normal levels of total serum protein and serum albumin. However, α1-acidglycoprotein, the major verapamil-binding serum protein, and α2-globulin were not analyzed. Also not analyzed were free DVPM concentrations. It has been found previously that rather moderate differences in the concentration of α1-acid glycoprotein can have significant effects on the ability of verapamil to overcome MDR (22). One way to control for variations in serum protein concentrations and for the presence of other compounds that may affect Pgp-inhibiting activity would be to spike the baseline patient samples in vitro with the chemosensitizer of interest and then to compare this particular dose-effect curve with that of the coprocessed, pooled serum controls spiked with the chemosensitizer. Any deviation found could be used then to normalize the activity produced by the chemosensitizer-containing patient sample. In the present study, this approach was not applied. Finally, we coprocessed only DVPM-spiked serum as a control in the analyses of clinical samples. When nor-DVPM was found to be more potent than the parent compound in increasing R123 retention, all clinical samples had been assayed already. It should be noted, however, that the extent of interexperiment variations in results was quite similar for DVPM- and nor-DVPM-spiked serum when analyzed in parallel. Thus, we felt able to apply the normalization coefficient that was calculated for DVPM to data for nor-DVPM also.

Preliminary data have been reported on the use of drug accumulation assays for analysis of chemosensitizer-containing patient serum, using either radiolabeled Pgp substrates or unlabeled anthracyclines for analysis by flow cytometry (13-15). In these studies, patient sera containing quinine and Cremophor EL, respectively, were found to be able to increase drug accumulation. The reported levels of bioassy activity were low and, thus, in agreement with the low activities on drug accumulation observed in the present studies. Irrespective of activity levels, drug retention or efflux seems to be the better test for Pgp function compared with the rather multifactorial phenomenon of drug accumulation. Thus, retention assays seem to be more appropriate for ex vivo analysis of chemosensitizer activity. Importantly, when analyzing the effects on drug retention, cells can be loaded with the indicator agent under standardized experimental conditions, and exposures to the index drug and chemosensitizer-containing serum can be separated. The R123 retention assay, therefore, allows straightforward interpretation of chemosensitizer effects on Pgp function.

In some clinical chemosensitizer studies, growth inhibition assays were applied to analyze ex vivo chemosensitizer activity (9-12). The tested agents were toremifene, amiodarone, S 9788, 0.58 1.05 1.647 2.841 4.487 1.465 1.124 3.94 4.43
10 0.95 1.44 1.794 3.249 5.043 1.638 1.095 3.58 3.92
11 1.26 1.80 1.928 3.679 5.607 1.725 1.118 6.86 7.67
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13 0.75 1.47 1.713 3.283 4.996 1.527 1.122 3.47 3.89
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16 1.12 1.60 1.867 3.340 5.301 1.761 1.060 3.60 3.82

Table 1 Plasma levels and bioassay activity of DVPM in patient samples

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<td>Database</td>
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aDVPM-spiked serum controls in particular analyses.
NC normalization coefficient, obtained by dividing theoretical activity of DVPM (a) by DVPM (b).
None of the coprocessed serum samples taken prior to administration of DVPM showed activity when compared with pooled human serum controls.
and PSC 833, and in all these studies, the chemosensitizer-containing patient samples were found capable of reducing resistance in Pgp-positive cell lines to a small extent. The number of ex vivo analyses reported in these studies was usually small, and procedures of assay validation have not been described. Furthermore, various cell lines used in these studies were reported to require heat inactivation of serum samples to allow adequate growth. Heating of drug-containing serum, however, is of some concern, because it may alter protein binding or the structure of a compound.

We recognize that activity by chemosensitizers on Pgp-mediated R123 efflux does not necessarily mean clinical activity on the Pgp-mediated transport of cytotoxic agents. Moreover, the increase in R123 retention produced by the patient samples in this study was relatively low considering the high degree of R123 effluxed by the 8226/DOX6 cells. Thus, the clinical relevance of this type of data remains to be determined. For the time being, however, an assay that is asking the simple question whether chemosensitizer-containing patient serum is capable of inhibiting Pgp function seems to provide meaningful information. And for such an assay, R123 seems to be an appropriate probe. R123 has been found to bind directly and specifically to Pgp, and binding was inhibited by drugs such as verapamil (26). R123 has been demonstrated to be an excellent molecular probe for analysis of Pgp function (27, 28) and has been used frequently to assess Pgp function in clinical tumor samples (29-33). Furthermore, R123 has been used to search for Pgp substrates in the National Cancer Institute drug screen, and its efflux pattern has been found to predict such substrates (34).

The R123 retention assay described in this report is inexpensive, rapid, and easy to use, does not involve radioactivity, and can be applied readily by routine laboratories that use flow cytometry. If the assay is used with the goal of assessing only Pgp-inhibiting activity of chemosensitizer-containing patient serum, then a limited number of controls is required, e.g., pooled human serum spiked with a single chemosensitizer concentration known to be active in the assay. If the goal is to use the bioassay activity to estimate blood levels of chemosensitizers, then a standard curve, derived from serum controls spiked with an appropriate number of graded concentrations of the chemosensitizer of interest, is required. It should be noted that direct determination of drug blood levels is usually expensive and laborious and often has to be carried out by laboratories that have particular expertise and experience in these types of analyses. Thus, analyses of chemosensitizer blood levels will be difficult to do as soon as agents are used in studies that are not supported by the pharmaceutical industry.

In the present study, nor-DVPM levels were generally higher than those of DVPM (mean, 1.6-fold; range, 1.08-2.02-fold). This is in contrast to previously reported data from studies using racemic verapamil as a chemosensitizer, in which the blood levels of norverapamil were lower than or equal to those of the parent compound (35-37). Recently, however, two trials have been published that used oral DVPM for overcoming chemotherapy resistance in patients with drug-refractory malignant lymphomas and renal cell carcinoma, respectively (38, 39). In both studies, trough levels of nor-DVPM were approximately 2-fold higher than those of DVPM, which is similar to our observations. These data seem important, because norverapamil has been found to have only 20% of the cardiovascular activity of the parent compound, and this when using racemic verapamil (40). Because the D-enantiomer of verapamil has been demonstrated to have 5-10-fold lower cardiac activity than the S-enantiomer (41), nor-DVPM can be speculated almost to lack cardiovascular effects. Along the same lines, our observation that nor-DVPM had a significantly higher molar potency than the parent compound in increasing R123 accumulation and retention in the 8226/DOX6 cell line could be of interest. The two compounds have been described previously as being equipotent in overcoming doxorubicin resistance in a murine tumor cell line (42). Apart from that single observation, however, no data have been published on their comparative MDR-reversing activities, and such studies seem warranted.

The R123 retention assay seems to be a sensitive, reproducible, rapid, and easy-to-use method for ex vivo analysis of the ability of chemosensitizer-containing patient serum to inhibit Pgp function, and this bioassay may prove useful for monitoring clinical chemosensitizer treatment of cancer patients.
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Sensitive and rapid bioassay for analysis of P-glycoprotein-inhibiting activity of chemosensitizers in patient serum.

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