RAPID FUNCTIONAL ASSAY FOR THE DETECTION OF MULTIDRUG-RESISTANT CELLS USING THE
FLUORESCENT DYE RHODAMINE 123

To the Editor:

Recently, Berman et al examined the effect of tamoxifen on two cell lines that display the multidrug-resistant (MDR) phenotype. Making use of the fluorescent properties of anthracyclines they described a rapid flow cytometric assay for the determination of intracellular daunorubicin (DNR) accumulation and retention. Such a technically simple assay may offer the possibility to predict
MDR in human tumor samples as it gives accurate information concerning the function of P-glycoprotein and overcomes problems associated with immunohistochemical or molecularbiologic methods.\textsuperscript{2,3}

We evaluated the intracellular uptake and retention of the fluorescent dye rhodamine 123 (R-123) in several MDR cell lines and their sensitive counterparts by using flow cytometry. After incubation with R-123 (50 to 200 ng/mL), time-dependent accumulation (at time 0, 15, 30, and 60 minutes; expressed as mean channel fluorescence) was determined in drug-sensitive (CCRF-CEM, KB-3-1) and -resistant (CCRF VCR1000, CCRF ADR5000, KB-8-5, KB-Colchicin) cell lines alone as well as in combination with verapamil. CCRF VCR1000, ADR5000, KB-8-5, and KB-C cells clearly accumulated less amounts of R-123 when being compared with the parental KB-3-1 and CCRF-CEM cells. The addition of verapamil (10 μmol/L) led to significantly increased intracellular R-123 levels in the resistant but not sensitive cell clones (Fig 1). Equivalent results were obtained in efflux experiments when cells were preloaded with R-123 for 1 hour, resuspended in drug-free medium and thereafter measured at time 0, 15, 30, and 60 minutes. Furthermore, in dilution experiments as few as 1% resistant KB-8-5 cells could be detected within a population of sensitive KB-3-1 cells.

In comparing R-123 with DNR, accumulation as well as retention studies proved R-123 as the more sensitive measure of MDR (eg, the discrimination between KB-8-5 and KB-3-1 cells was four times as accurate). Moreover, dual-fluorescence experiments were performed with both dyes. For this study peripheral blood samples of healthy donors were used. Adequate compensation between R-123 (green fluorescence, maximum 534 nm) and phycoerythrin (PE)-labeled antibodies\textsuperscript{4} allowed accurate measurement of both signals (Fig 2). In contrast DNR (red fluorescence, maximum 585 nm) showed a strong overlapping emission spectrum into the fluorescein isothiocyanate (FITC) signal that could not be compensated by changes in the instrument setting.

For the functional evaluation of MDR cells by laser flow cytometry we suggest the use of the fluorescent dye R-123 for the following reasons: (1) Existence of cross-resistance between anthra-

cyclines and R-123 in cells that display the MDR phenotype.\textsuperscript{5} (2) Assessment of cellular uptake/efflux with R-123 is a more sensitive measure of MDR than DNR. (3) Feasibility of dual fluorescence staining with PE-conjugated antibodies. Considering the heterogeneous nature of clinical samples (eg, bone marrow) this approach may be of special importance. (4) No cytotoxicity at the concentrations used.\textsuperscript{6} Our results warrant further investigation with R-123

\textbf{Fig 1.} R-123 (100 ng/mL) uptake assessed by flow cytometric analysis in CCRF-CEM, CCRF VCR1000, and CCRF ADR5000 cells with/without 10 μmol/L verapamil (V).

\textbf{Fig 2.} Double fluorescence data of intracellular R-123 (green fluorescence) and surface CD14/Leu-M3 staining (red fluorescence) showing monocytes at time 1 (A) and 90 (B) minutes. Peripheral blood of a healthy donor was used.
for the prediction of MDR in cell lines and subsequently in clinical samples (currently in progress).

CHRISTOF LUDESCHER
CLAUS GATTRINGER
JOHANNES DRACH
Department of Internal Medicine
JOHANN HOFMANN
HANS GRUNICKE
Department of Clinical Biochemistry
University of Innsbruck
Innsbruck, Austria

REFERENCES


RESPONSE

Ludescher et al describe a functional assay for detecting multidrug resistance (MDR) using the fluorescent dye rhodamine 123 (R123). In this system they show that R123 has a modest degree of uptake in three MDR cell lines; the addition of verapamil markedly enhances its uptake in two of these three cell lines, as shown in Fig 1. The investigators suggest that the R123 uptake and retention pattern is similar to that shown by daunorubicin (DNR) in two other MDR cell lines. The investigators state that R123 accumulation in MDR cells is four times more sensitive than is DNR accumulation (however, no comparative data is shown) and suggest that the R123 fluorescent assay system may prove useful in predicting the presence of MDR cells in vitro and in clinical samples.

A functional assay that would predict the MDR phenomenon in clinical samples is an interesting concept but one that may prove difficult to interpret. It has been well documented that P-glycoprotein is present on normal cells in the colon, kidney, pancreas, trachea, and capillary endothelial cells lining certain areas of the brain; presumably, these cells would stain positive in the R123 assay. It is not clear from the above report whether R123 uptake correlates with the presence of P-glycoprotein and it would have been of benefit to have simultaneously stained the cell lines with a monoclonal antibody directed against P-glycoprotein such as C2194 or HYB 241. Nonetheless, further investigation using R123 uptake assay is of interest and we await the detailed studies from this group.

ELLIN BERMAN
Leukemia Service
Memorial Sloan-Kettering Cancer Center
New York, NY

REFERENCES


Rapid functional assay for the detection of multidrug-resistant cells using the fluorescent dye rhodamine 123 [letter; comment]

C Ludescher, Gattringer, J Drach, J Hofmann and H Grunicke