and P-gp and was only 2- to 3-fold more resistant to epirubicin (EPI) and DOX but almost as sensitive to daunorubicin (DAU) as CCRF-CEM, a sensitive, parental line. The induction of MDR1 expression by three anthracyclines, DOX, DAU and EPI, was quantitatively analyzed by scanning Northern blots on a phosphorImager using ImageQuant software (Molecular Dynamics). The ratio of MDR1 to the internal RNA (actin) control was expressed as an average ± standard deviation and statistically analyzed using the Student's *t*-test. A significant increase (2-fold) in expression of MDR1 was noted within 4 h of exposure to 1.5 μg/ml of DAU or EPI. After an 8 h exposure to 1.5 μg/ml of DOX, DAU or EPI, there was a 3- to 4-fold increase in MDR1 expression in the CEM/A7R line, although neither vinblastine (VBL) nor vincristine (VIN) had any effect on MDR1 expression. With increasing concentrations of DAU and EPI in a fixed 24 h time period, MDR1 expression (relative to the RNA control) increased although a biphasic response was seen. Based on the binding of MRK-16, an increase in P-gp levels was seen in the CEM/A7R line after exposure to 1 μg/ml of DAU or EPI. These data suggest that anthracyclines differentially modulate upregulation of MDR1 expression in the CEM/A7R cell line. In addition, the rapid increase in MDR1 expression after a short period of exposure to DOX, DAU or EPI (findings not previously reported in human cell lines) suggests that induction of MDR1 expression may have an important role in the 'selection' of drug resistant tumors.

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Increased ecto-γ-glutamyltransferase activity and susceptibility to apoptotic cell death in a MDR+ T-cell line

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The enzyme γ-glutamyltransferase (γ-GT) catalyzes the first step in the extracellular transpeptidation of the cellular glutathione into amino acid intermediates which are subsequently transported across the cell membrane. Many studies have shown that γ-GT was elevated in various tumors, enhanced in dexamethasone-induced apoptotic CEM cells and found modulated in drug resistance. The γ-GT activity and its kinetic properties were analyzed in two human T-lymphoblastoid cell lines, one sensitive (CCRF-CEM cells) and the other resistant (VBL-100 CEM cells) to vinblastin. Resistant VBL-100 CEM cells, unlike the CCRF-CEM subclone, reacted with monoclonal antibodies directed against external epitopes of the MDR1 gene product, P-gp and exhibited a γ-GT activity higher (3.6 nmol/min) than the sensitive subclone (2.4 nmol/min) devoid of P-gp. In contrast, substrate affinity (Michaelis–Menten analysis) was found similar (K_m = 1.0 mM). Acivicin, a specific inhibitor of γ-GT activity, induced a dose-dependent growth inhibition of both cell lines, but its apoptotic effect at 10^{-6} M was more marked than that of dexamethasone, a known inducer of apoptosis in CEM cells. When compared to CCRF cells, the VBL-100 resistant subclone showed a higher degree of γ-GT inhibition by acivicin and also an increased level of apoptosis. The results suggest that drug-resistant VBL-100 CEM cells, bearing the MDR1-encoded efflux system P-gp, also exhibit an increased membrane γ-GT complex which might be related to an active influx system and to an increased susceptibility to apoptotic cell death.

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Characterization of altered intracellular drug distribution in LZ cells and primary human breast tumors

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Sequestration of drugs in vesicles and nuclear drug exclusion are thought to contribute to cellular drug resistance but are currently poorly understood. This study characterized these phenomena in an established series of multidrug resistant cells, designated LZ, and in primary human breast tumors. In LZ cell lines exhibiting different levels of resistance to doxorubicin (DOX), the number of drug sequestering vesicles observed following a 2 h DOX exposure correlated with the level of drug resistance achieved but not with the level of P-glycoprotein present in the cell membrane (Western blots), level of gene expression (mRNA-dot blot hybridization) or level of MDR1 gene amplification (DNA-dot blot hybridization). A 50% inhibition in number of vesicles (fluorescent microscopy quantification) occurred with X-ray (10 Gy) pretreatment prior to DOX and DOX nuclear exclusion was reversed but DOX efflux was not altered (spectrophotometric method). This radiation reversal effect exhibited a dose-response. In contrast, verapamil pretreatment (100 μM) did not affect the number of vesicles seen with a 2 h DOX exposure. Laser scanning confocal microscopy studies of cells from untreated primary human breast tumors exposed 2 h to 10 μg/ml DOX detected vesicular drug sequestration in five samples, nuclear drug exclusion in four and both phenomena simultaneously in two. No correlation was seen between MDR1 gene amplification/expression (dot blot hybridization) and vesicular drug sequestration. Thus, nuclear drug exclusion and vesicular drug sequestration may represent distinct mechanisms contributing to resistance. In summary, drug sequestration and nuclear drug exclusion contribute to cellular drug resistance, occur in human clinical tumor samples, and can be partially reversed by X-ray treatment.

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