showed progressive decrease in ER levels, accompanied by a progressive increase in EGF-R, and P-gp levels with increased tumorigenicity of the cell line; i.e. initially the parent cells are weakly tumorigenic and sensitive to both T and Adr, but as subculturing continues, there is significant increase in cellular tumorigenicity and resistance to higher concentrations of T and Adr. These observations suggest that ER levels vary inversely with EGFR and PG levels, and these changes increase tumor cell aggressiveness with the ability to grow in presence of T and Adr.

1. Hakim AA. *Surg Forum* 1975; 26: 175–7.
2. Hakim AA. *Experientia* 1976; 82: 1057–9.
3. Hakim AA. *Expt Cell Biol* 1979; 47: 332–50.
4. Hakim AA. *Proc Expt Biol Med* 1989; **185**, 158–76.
5. Hakim AA. *J Surg Oncol* 1989; 76: 329–31.

6
Collateral sensitivity of P-glycoprotein overexpressing cell lines to dexniguldipine–HCl: effects on DNA replication, cell cycle and the dCT pool

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Dexniguldipine–HCl (DNIG), an enantiomeric-pure dihydropyridine, potently modifies P-glycoprotein (P-gp) mediated multiple drug resistance (MDR) at submicromolar concentrations *in vitro* via direct binding to P-gp. Clinical trials as an MDR modulator have been initiated. In addition, DNIG showed selective anti-proliferative activity against some experimental tumors *in vitro* and *in vivo* which has been attributed to its PKC inhibitory or calmodulin antagonistic properties. Therefore, phase II trials of DNIG as an anti-cancer agent are under way. The anti-proliferative quality of the compound on a series of P-gp overexpressing MDR sublines and the corresponding parental tumor cell lines was analyzed. Applying a 72 h tetrazolium based colorimetric MTT-assay, IC_{50} values of about 5 μM were usually obtained. A collateral sensitivity, however, of several P-gp overexpressing cell lines was seen revealing IC_{50} values of about 1 μM. In the case of the highly P-gp expressing cell line CCRF ADR 5000, and the parental human T-lymphoblastoid cell line CCRF-CEM the effect of DNIG was further examined, i.e. by DNA fiber autoradiography, flow cytometry and desoxynucleotidetriphosphate pool measurements. At micromolar concentrations DNIG induced exclusively in the MDR subline a stop of replication forks, an arrest in the G_1 or early S phase of the cell cycle, and a selective depletion of the cellular dCTP-pool. The involvement of P-gp mediated functions in this phenomenon remains to be investigated.

7
Lack of P-glycoprotein involvement in the transport of VP-16 in multidrug resistant tumor cells

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Although overexpression of P-glycoprotein (P-gp) is one of the major mechanisms of multidrug resistance (MDR), additional mechanisms can occur concurrently. The role of P-gp in the transport of anthracylcines and *Vinca* alkaloids has been investigated in detail. However, the transport of etoposide (VP-16) in MDR cells is not well characterized. To examine the role of P-gp in the resistance to (VP-16), the cytotoxicity, cellular accumulation/retention, efflux, and nuclear uptake of VP-16 in several P-gp positive multidrug resistant cell lines were investigated. The effects of MDR modulators R-verapamil (R-VPM), dipyridamole (DP) and metabolic inhibitor sodium azide (NaN_3) on the above mentioned parameters were also examined. MDR cell lines used were: (1) breast carcinoma MDA-A1R (derived by treating parental MDA-MB-231 cells with doxorubicin); and (2) KB-GRC1 (derived by transfection of the MDR1 gene in drug-sensitive KB-3-1 cells). In clonogenic assay, R-VPM (10 μM) caused a 5-fold and a 4.5-fold reversal of the resistance to VP-16 in MDA-A1R and KB-GRC1 cell lines respectively. However, R-VPM did not significantly ($p < 0.05$) alter [^{3}H]-VP-16 efflux. NaN_3 did not alter [^{3}H]-VP-16 efflux either but significantly ($p < 0.05$) increased the [^{3}H]-VP-16 levels in MDA-A1R (6-fold) and KB-GRC1 cells (7.5-fold). The results are shown below:

Cell lines	R-VPM		Sodium azide	
	uptake	efflux	uptake	efflux
MDA-MB-231	↑ (1.5-fold)	↔	↑↑ (3-fold)	↔
MDA-A1R	↔	↔	↑↑↑ (6-fold)	↔
KB-3-1	↔	↔	↑↑ (3-fold)	↔
KB-GRC1	↑ (1.7-fold)	↔	↑↑↑ (7.5-fold)	↓

It appears that VP-16 efflux is not a good substrate for P-gp. However, the process of intracellular binding and/or distribution appears to be energy dependent.