8
Photoaffinity labeling of P-glycoprotein (P-gp) with a photoreactive analog of dexniguldipine–HCl: comparison with [^{3}H]azidopine

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Dexniguldipine–HCl (B8509-035, DNIG) is a very potent compound for reversal of MDR and is now in phase II clinical trials. The ^{3}H-labeled azido analog of DNIG ([^{3}H]B9209-005) was developed to identify the target proteins. The biological activity of B9209-005 with respect to MDR reversal remained almost unchanged. Applying the tetrazolium based colorimetric MTT-assay for monitoring cell proliferation, or a rhodamine-123 flow cytometry accumulation assay for measuring P-gp transport function, B9209-005 shows similar activity to DNIG and > 10-fold potency compared to azidopine. Photoaffinity labeling of membranes prepared from multidrug resistant P-gp overexpressing (ADR-5000) and parental T-lymphoblastoid cell lines (CCRF-CEM), were labeled with increasing concentrations of [^{3}H]B9209-005. In contrast to membranes from the sensitive cell line, two proteins with apparent molecular weights of 170 and 95 kD were specifically labeled in membranes from the cell line ADR-5000. They were identified as P-gp and a P-gp proteolytic fragment by immunoprecipitation with the mAb C219. The specificity was established by competitive blocking of photolabeling with nonradioactive DNIG or with anti-cancer drugs, e.g. vinblastine. 'Scatchard analysis' of the P-gp labeling revealed one specific binding site. These results indicate that DNIG reverses the MDR phenotype by binding directly to P-gp. In contrast to [^{3}H]B9209-005, [^{3}H]azidopine specifically labeled another proteolytic fragment of P-gp with a molecular weight of 55 kD. The different labeling pattern of [^{3}H]azidopine and [^{3}H]B9209-005 can be explained by the different position of the azido-group in the photoligands. The dexniguldipine-derivative [^{3}H]B9209-005 bearing the azido-group on the dihydropyridine moiety and not in the side chain (as is the case for azidopine), thus appears to be well suited for the analysis of the binding sites on P-gp.

9
Doxorubicin, daunorubicin and vinblastine are transported by a glutathione conjugate transporter

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A glutathione conjugate transport mechanism widely expressed in human tissues, designated dinitrophenyl-S-glutathione ATPase (Dnp-SG ATPase), has previously been shown to catalyze ATP hydrolysis in the presence of several amphiphilic compounds other than glutathione conjugates.[1] The present studies have isolated this transporter from human erythrocyte and lung and have shown that doxorubicin (DOX) and its metabolites stimulated ATP-hydrolysis by the purified transporter in a saturable manner (K_m for DOX 1.2 and 2.8 μM for the lung and erythrocyte enzyme respectively). The activity was immunoprecipitable by antibodies specific for Dnp-SG ATPase. A new method for studying transport of DOX was devised and standardized in erythrocyte inside-out vesicles (IOVs) using a rapid membrane filtration method for separating free from IOV associated DOX. Using this method, it was established that DOX was transported into IOVs in a manner saturable with respect to DOX (K_m = 1.8 μM, V_{max} = 280 pmol/min/mg protein) and ATP concentration (K_m = 1.9 mM), linear with respect of IOV protein and time points up to 10 min, sensitive to orientation of vesicles, sensitive to osmolarity and dependent on temperature (energy of activation 13 kcal/mol). Transport of daunomycin and vinblastine was also observed. Anti-Dnp-SG ATPase antibodies inhibited transport. Reconstitution of purified Dnp-SG ATPase into erythrocyte IOVs resulted in increasing transport rate of DOX in a manner linear with respect to amount of purified protein added. The transport was competitively inhibited by glutathione conjugate of ethacrynic acid and verapamil and non-competitively by cyclosporine. These results demonstrate that a shared mechanism exists for transport of glutathione conjugates and amphiphilic cytotoxins which are known to be substrates of the P-glycoprotein and suggest that glutathione conjugates may be useful in modulating anthracycline chemotherapy.[2]

1. Singhal SS *et al. FEBS Lett* 281: 255–7.
2. Awasthi S *et al. J Clin Invest* 1994; 93: 958–65.

10
Rapid but differential upregulation of MDR1 expression by anthracyclines in a classical drug resistant cell line

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Studies were carried out in a 'revertant' human multidrug resistant (MDR) cell line CEM/A7R derived from the low level doxorubicin (DOX) resistant leukemia cell line CEM/A7. The CEM/A7 line overexpresses P-glycoprotein (P-gp), although no gene amplification has been demonstrated. Compared to the CEM/A7 line, the revertant CEM/A7R line expressed very low levels of MDR1 mRNA