found for the hepatoma subgroups (40.2 ± 18.2% versus 56.1 ± 9.6%). The initial dye efflux velocities were significantly different for the responders and non-responders of both tumor groups (pancreas: 6.1 ± 1.8% versus 0.9 ± 0.6% × 10^{-2} s and hepatoma: 4.0 ± 2.2% versus 8.5 ± 2.3% × 10^{-2} s).

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Photosensitization of vinblastine by riboflavin

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In studies aimed at experimental MDR reversal by vinblastine photoderivatives, poor reproducibility of vinblastine cytotoxicity tests *in vitro* has been encountered. Contrary to a widely held belief, vinblastine itself was found to be completely insensitive to the light wavelengths and intensities commonly prevailing in such tests. However, we found that visible light triggers rapid riboflavin-mediated photoreactions with vinblastine. The photoreactions alter the absorption spectrum and result in the formation of chromatographically separable degradation products of vinblastine. As exemplified on both chemosensitive and MDR mouse ascites cells, neither sensitivity nor resistance to vinblastine can be reliably determined as long as riboflavin-mediated photoreactions prevail. Under conditions chosen to prevent such reactions, IC_{50} values of 1.02 ± 0.22 nM and of 18.5 ± 3.42 nM vinblastine were determined in sensitive and resistant cells, respectively. Corresponding values from series of experiments without precautions fluctuated in the ranges from 3 to 30 nM and from 15 to 360 nM. Riboflavin, an obligatory component of cell culture media, photosensitizes some other cytostatic drugs as well. Its photoactivation therefore needs to be fully prevented, especially in experimental and clinical protocols for MDR detection.

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Subcellular immunolocalization of P-glycoprotein in multidrug resistant cell lines

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The lack of nuclear accumulation of doxorubicin (DOX), a distinctive feature of multidrug resistant (MDR) cells, can be easily detected by the natural fluorescence of this drug. Cytofluorometry and confocal microscopy were used to study DOX subcellular distribution in the human osteosarcoma cell line U-2 OS and in its MDR variant U-2 OS/DX^{580}. As previously shown in other MDR cell lines, DOX fluorescence was stable in the nucleus of sensitive cells, whereas in resistant cells the drug could not achieve detectable nuclear levels due to an energy-dependent mechanism. Continuous exposure to verapamil, a known inhibitor of P-glycoprotein (P-gp) activity, was necessary to obtain a complete reversal of resistance in MDR cells and to achieve DOX-induced cytotoxic effects comparable to those of sensitive cells. In order to investigate the relationship between subcellular localization of P-gp and DOX extrusion from the nucleus, we have evaluated the expression of this protein in U-2 OS/DX^{580} and in two other MDR cell lines (SW948-R-300 and LoVo-R-100) by confocal microscopy and immunoelectron microscopy, using a panel of antibodies (JSB-1, C219 and MRK16). Compared to their corresponding sensitive cells, MDR cell lines U-2 OS/DX^{580} and LoVo-R-100 showed higher levels of P-gp expression, not only at the plasma membrane and inside the cytoplasm, but also in the nucleus. SW948-R-300 cells, featuring increased amounts of MDR1 mRNA but lacking P-gp expression at the cell surface, showed a higher P-gp immunolabeling only in the nucleus and in the cytoplasm. The nuclear localization of P-gp in different MDR cell lines, confirmed also by studies on isolated nuclei and nuclear matrices, raises new questions about the functional role of this protein.

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Mouse monoclonal antibodies directed against a recombinant peptide homologous to human MDR3 membrane P-glycoprotein.

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MDR3 is a P-glycoprotein that seems to be overexpressed exclusively in leukemias and lymphomas of B-cell origin.[1] The present study analyzes MDR3 expression in a series of B-cell non-Hodgkin's lymphomas (NHL) of low, intermediate and high grade malignancies, using a RNase protection assay. MDR3 expression in untreated NHL had a mean of 1.71 units, SD = 2.49 ranging from 0.2 units to 9 units (human liver: 30 units), compared to MDR1 with a mean of 3.52 units, SD = 4.47 ranging from 0.2 units to 20 units. In order to investigate the possible significance of MDR3 expression in NHL we have produced specific monoclonal antibodies directed against human MDR3 P-gp. For this goal we used a recombinant protein expressed in bacterial *Eschericia coli* strains. The recombinant peptide contained an amino acid sequence similar to the amino acids 637–690 in the human MDR3. For production of the recombinant protein a PCR fragment was synthesized with specific primers and a cDNA