J Cancer Res Clin Oncol (1989) 115: 17–24

Journal of Cancer Research and Clinical Oncology

© Springer-Verlag 1989

Induced multidrug resistance in murine leukemia L1210 and associated changes in a surface-membrane glycoprotein

Manfred Volm, Mihaly Bak Jr. *, Thomas Efferth and Jürgen Mattern

German Cancer Research Center, Institute of Experimental Pathology,
Im Neuenheimer Feld 280, D-6900 Heidelberg, Federal Republic of Germany

Summary. The aim of this study was to find out whether only resistant cells of the "multidrug-resistant" phenotype show the described changes of plasma membrane glycoprotein (170 kDa) or whether resistant cells that do not express this phenotype reveal corresponding results. Doxorubicin-resistant ($L1210_{dox}$) and daunorubicin-resistant L1210 ascites tumor cells ($L1210_{dnr}$) (multidrug-resistant tumor cells) were therefore compared with cytosine-arabinoside-resistant ($L1210_{AraC}$) and cyclophosphamide-resistant L1210 ascites tumor cells ($L1210_{ctx}$) (not multidrug-resistant tumor cells). The resistant cell lines were generated in vivo in tumor-bearing mice and the resistance to cytostatic agents was evaluated in vivo and in vitro. Using the accumulation assay with rhodamine-123, the multidrug resistance can be detected. In order to determine alterations in the plasma membranes we used the monoclonal antibodies 265/F4 and C219, which were prepared against the membrane glycoprotein P170 (170 kDa) in colchicin-resistant Chinese hamster ovary cells. The results demonstrate that $L1210_{dox}$ and $L1210_{dnr}$ tumor cells show an intense immunostaining by the streptavidin/biotinylated-peroxidase-complex method and by the streptavidin/biotin/phycoerythrin immunofluorescence method. In contrast no specific immunostaining was observed in parental (sensitive) and $L1210_{AraC}$ or $L1210_{ctx}$ tumor lines. The results were confirmed by immunoblotting. To determine whether multidrug-resistant DNA sequences were expressed in the multidrug-resistant tumor cells, Northern blots with RNA od sensitive and resistant cells were performed using the clone pcDR1.5. Elevated RNA levels were detected only in resistant cells with the multidrug-resistant phenotype. Thus, the results of this study demonstrate that only resistant cells with the multidrug-resistant phenotype show an increased expression of the membrane 170-kDa glycoprotein.

Key words: Multidrug resistance – Leukemia L1210 – Membrane glycoprotein – Rhodamine-123 – Monoclonal antibody

Introduction

Tumors that are initially responsive to chemotherapy can develop resistance during treatment. Clinically this is characterized by short periods of remission and failure to respond to subsequent therapy. Frequently, resistant tumor cells can acquire a cross-resistance to a wide range of compounds that have no obvious structural or functional similarities, e.g. alkaloids, anthracyclines and antibiotics (Biedler et al. 1983; Riordan and Ling 1986). This phenomenon has been designated as pleiotropic or multidrug resistance (MDR). Multidrug resistance seems to be associated with a decreased net cellular concentration of the drugs involved and has been attributed to alterations in the plasma membrane (Danø 1973; Juliano and Ling 1976; Skovsgaard 1978; Beck et al. 1979). In addition, the multidrug-resistant monolayer cells often contain amplified genes and the level of expression of the genes is increased in drug-resistant cells (Roninson et al. 1984; Riordan et al. 1985).

The aim of this study was to find out whether only resistant cells of the MDR phenotype show the changes of plasma membranes described, or whether resistant cells not expressing the MDR phenotype reveal corresponding results. Therefore, we established resistant L1210 leukemia cells, which express the MDR phenotype (doxorubicin-resistant and daunorubicin-resistant L1210 ascites tumor cells), and L1210 ascites tumor cells, which do not express this

* Guest Scientist from the National Institute of Oncology, Budapest Hungary

Offprint requests to: Manfred Volm

phenotype (cytosine-arabinoside-resistant and cyclophosphamide-resistant L1210 ascites tumor cells). The tumor cell lines were generated in vivo in mice – an approach which might be more analogous to the clinical development of drug resistance.

Materials and methods

Cytostatics. The following were used: doxorubicin (Adriblastin, Farmitalia, Freiburg i. Br., FRG) and daunorubicin (Daunoblastin, Farmitalia, Freiburg i. Br., FRG); cytosine arabinoside (Alexan, Mack, Illertissen, FRG); cyclophosphamide (Endoxan) and Asta Z-7557 (Mafosphamide, Degussa Pharma-Gruppe, Asta Bielefeld, FRG); actinomycin D (Lyovac Cosmegen, MSD Sharp and Dohme, New Jersey, USA).

Animals. Female NMRI mice (from the Breeding Center, Hannover, FRG), weighing between 20 g and 25 g, were maintained under standard conditions: 21 °C room temperature, 65% humidity, 12 h light, 12 h darkness, Macrolon cages, water and Altromin standard diet (Altrogge, Lage, FRG) ad libitum.

Tumors. The leukemia L1210 cells were grown intraperitoneally (i.p.) in ascites form and were transplanted at 7-day intervals (1.5×10^7 cells/mouse). The total cell count increased exponentially in the first few days, reached a maximum at day 11 and showed only minor changes thereafter.

Development of resistant tumor cell lines. Animals bearing L1210 cells were treated with cytostatics i.p. weekly. Doxorubicin and daunorubicin were applied at a concentration of 2 mg/kg body weight. Cytosine arabinoside was applied at a concentration of 120 mg/kg body weight and cyclophosphamide at a concentration of 100 mg/kg body weight weekly. This pretreatment was carried out for at least 20 passages. The doxorubicin-resistant tumor lines were designated as $L1210_{dox}$, the daunorubicin-resistant tumor line as $L1210_{dnr}$, the cytosine-arabinoside-resistant tumor line as $L1210_{AraC}$ and the cyclophosphamide-resistant tumor lines as $L1210_{ctx}$.

Detection of resistance to cytostatics in vivo. For the detection of resistance, L1210 cells grown in ascites form were injected i.p. in animals. The test was carried out by injecting groups of animals with either untreated (sensitive) or drug-pretreated (resistant) tumor cells. The drugs were injected i.p. on the 4th and 5th days after tumor transplantation. Half of the total dose was injected on each day. The effects of the drugs on the tumor cells were measured 2 days after the last injection. For this reason the tumor-bearing animals were killed and the ascites cells were removed by puncture and counted using a coulter counter.

Detection of the resistance by the short-term test. The short-term test was carried out by the method described earlier (Volm et al. 1985; 1988). Briefly, tumor cells were incubated with the corresponding cytostatic agents at different concentrations for 3 h at 37 °C. The corresponding radioactive precursors for nucleic acid were added during the last hour of incubation. Aliquots of the cell suspension were pipetted onto filter-paper discs, the acid-soluble radioactivity was extracted and the incorporated activity measured by scintillation counting.

Accumulation of rhodamine-123 in tumor cells. Ascites cell suspension (10 µl, about 10^4 cells) was pipetted onto microscopic slides and a final concentration of 5 µg/ml rhodamine-123 (R-123) was added in order to determine the accumulation of R-123. Propidium iodide monohydrate was used at a concentration of 35 µg/ml to stain nuclei of dead cells (dead cells must be excluded because they accumulate R-123 unspecifically). At intervals of 2 min all R-123-stained or unstained cells in a viewfield (about 80–120 cells) were counted. Data were collected at fluorescence emissions above 510 nm (R-123) and 580 nm (propidium iodide monohydrate).

Immunocytochemistry. For immunocytochemistry the biotin/streptavidin method was employed (Hsu et al. 1981; Bak et al. 1987). Briefly, tumor cells were suspended in Hank's salt solution containing 3% bovine serum albumin and centrifuged by Cytospin 2 (Shandon), resulting in a monolayer cell smear on the slides. After air drying, the cells were fixed in acetone. After incubation with normal sheep serum the primary monoclonal antibodies (265/F4 and C219) were applied (5–20 µg/ml) for 2 h. The preparation and characterization of these monoclonal antibodies have been described earlier (Kartner et al. 1985; Lathan et al. 1985). The antibodies were kind gifts from Dr. B. Lathan, Cologne, FRG and Dr. V. Ling, Toronto, Canada. The antibodies were prepared against the membrane glycoprotein P170 (170 kDa) in colchicine-resistant Chinese hamster ovary (CHO) cells. After washing, the cells were incubated with biotinylated sheep anti-(mouse Ig) second antibody (dilution 1:50, 30 min, Amersham) and after rewashing in phosphate-buffered saline, immunostaining was carried out with the streptavidin/biotinylated-peroxidase complex (Amersham) (dilution 1:100, 30 min). Peroxidase activity was visualized by 3-amino-9-ethylcarbazole/H_2O_2 (5–10 min). Counterstaining was performed with Mayer's haematoxylin. Parallel studies, substituting normal mouse serum for the primary antiserum, were used as controls. In addition, comparably treated sensitive and resistant CHO cells served as negative and positive reference controls.

Immunofluorescence. For immunofluorescence detection of glycoprotein P170 we used the recently developed streptavidin/biotin/phycoerythrin method. Primary and second antibody were applied as described above. Then the cytospin smears were incubated with biotinylated-streptavidin/phycoerythrin complex (Amerham, dilution 1:50, 40 min). After addition of a stabilizer for 20 min to prevent rapid fading of phycoerythrin fluorescence, the slides were dried and mounted. For fluorescence microscopy, excitation at 546 nm was used. Data were collected at fluorescence emissions above 580 nm.

Immunoblotting. The isolation of plasma membranes from sensitive and resistant L1210 cells was performed according to a slightly modified method of Riordan and Ling (1979). Tumor cells of each six animals were pooled and stored at −80 °C. For each cell line four different pools (24 different tumors) were analyzed. Cells were homogenized in the presence of a hypotonic buffer 15 m*M* Tris/HCl, pH 8; 1 m*M* $MgCl_2$; 1 m*M* $CaCl_2$; 1 m*M* phenylmethylsulfonyl fluoride). Homogenates were centrifuged at 800 *g* to pellet nuclei, at 4000 *g* to pellet mitochondria and subsequently at 40000 *g* to obtain the microsomal fraction. The microsomal fraction was stored at −80 °C. One-dimensional sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a slab gel apparatus according to Fairbanks et al. (1971). Protein concentration in the different protein extracts was determined according to the method of Bradford (1976). Immunoblotting was performed according to Towbin et al. (1979) and Lathan et al. (1985). After transfer, additional protein-binding sites on nitrocellulose were blocked for 2 h at 37 °C in NEH buffer (50 m*M* Hepes, pH 7.4, 5 m*M* Na_2EDTA, 150 m*M* NaCl) containing 3% bovine serum albumin, 0.25% gelatine, 0.05% Triton X-100. The nitrocellulose was then incubated at 37 °C for 1 h with monoclonal antibody (265/F4; concentration: 10 µg/ml) in a dilution buffer consisting of NEH buffer (with 1% bovine serum albumin, 0.25% gelatine, and 0.05% Triton X-100, pH 7.4). After washing with wash buffer (NEH buffer containing 0.25% gelatine and 0.05% Triton X-100) the nitrocellulose was incubated for 1 h at

37 °C with the second antibody: biotinylated sheep anti-(mouse immunoglobulin) (Amersham, dilution 1:200). After washing, labeling was carried out with the streptavidin/biotinylated-horseradish-peroxidase complex (Amersham; dilution 1:400, 30 min). After successive washes with wash buffer and substrate buffer (50 mM Hepes, 150 mM NaCl, pH 7.4) the nitrocellulose blots were incubated with 50 ml substrate buffer, 10 ml 4-chloro-1-naphthol (3 mg/ml methanol) and 50 µl H_2O_2 (10–30 min). The reaction was stopped by washing with water. Efficiency of the electrophoretic transfer was monitored by Ponceau staining of the gels. Sensitive and resistant CHO were used as additional controls.

Northern blotting. RNA was isolated from tumor cells by the guanidinium isothiocyanate method. For Northern blot analysis, RNA was separated by electrophoresis on formaldehyde gels and transferred to nitrocellulose membranes, which were subsequently hybridized with the ^{32}P-labeled pcDR1.5 probe (the probe was provided by Dr. Croop, Cambridge, Mass, USA). The isolation of the probe has been described earlier (Gros et al. 1986). Hybridization was carried out at 42 °C in a solution of 50% formamide, 6 × SSPE (10 mM sodium phosphate, pH 7.0; 0.18 M NaCl; 1 mM Na_2EDTA), 5 × Denhardt's solution, 1% SDS and 125 µg/ml denatured salmon sperm DNA. The membranes were washed several times with standard saline citrate, 1% SDS at 55 °C, dried and exposed with Kodak XAR-5 films at −70 °C.

Results

Detection of resistance in vivo

The L1210 ascites tumor cells preconditioned with doxorubicin (L1210$_{dox}$), daunorubicin (L1210$_{dnr}$), cytosine arabinoside (L1210$_{AraC}$) and cyclophosphamide (L1210$_{ctx}$) are more resistant to the correspond drugs in vivo than the parental (sensitive) cells (Fig. 1).

Detection of resistance and cross-resistance in vitro

An in vitro short-term test was also used to detect the resistance of the pretreated tumor lines and to define the degree of resistance. The basic feature of this test is the measurement of inhibition of incorporation of radioactive nucleic acid precursors into tumor cells after addition of cytostatic agents. The resistance of the pretreated cell lines could be confirmed in vitro (Fig. 2). The maximum degree of resistance was 45-

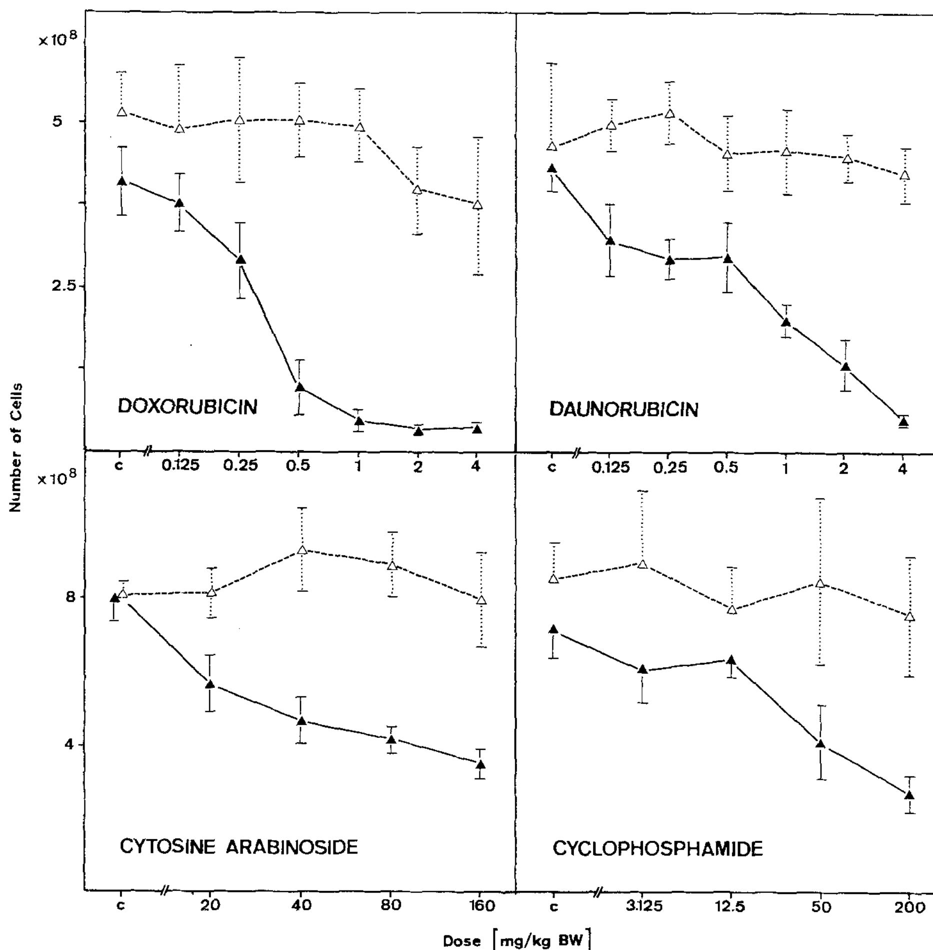

Fig. 1. Detection of resistance of preconditioned L1210 ascites tumor cells to different corresponding cytostatic agents in vivo. *Abscissa*, different dose levels of drugs (total doses are given). *Ordinate*, number of cells (each point represents mean value with confidence interval, $\alpha = 0.05$, from ten animals). Treatment was on the 4th and 5th days, determination of cell counts on the 7th day after tumor transplantation. ▲-▲, Sensitive parental tumor line; △⋯△, drug-pretreated tumor line

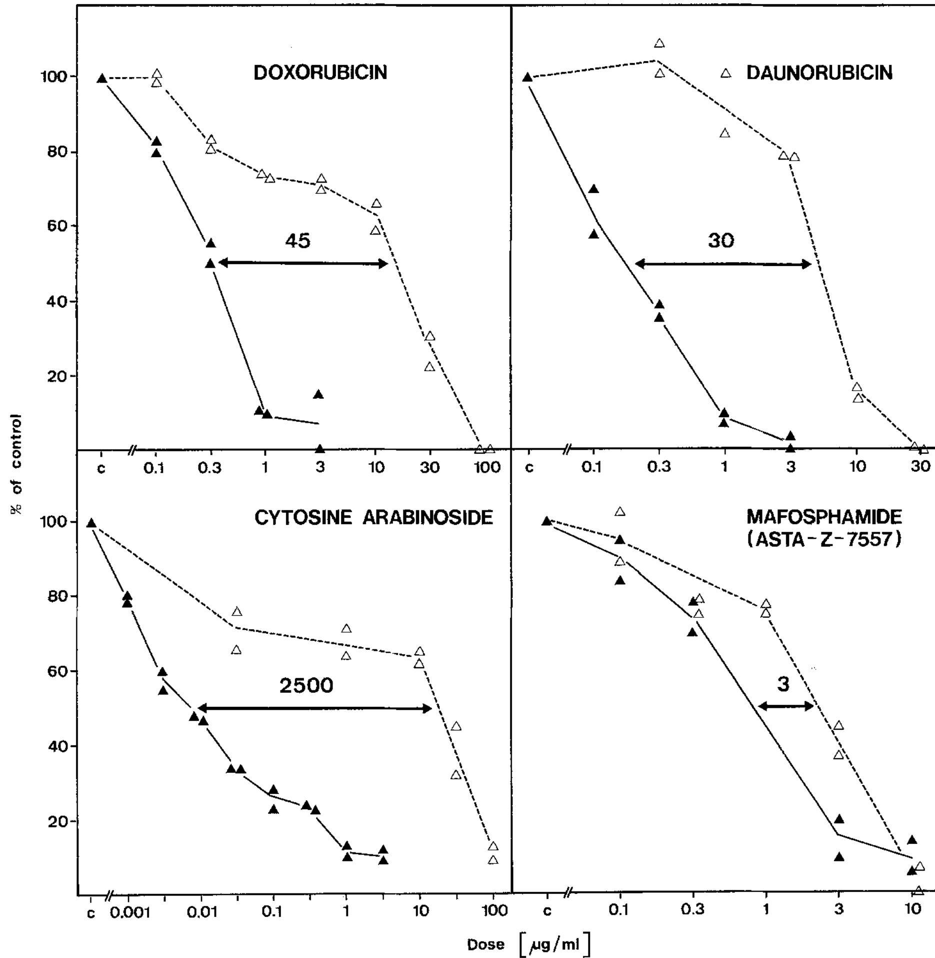

Fig. 2. Detection of resistance of pretreated L1210 tumor cells to different corresponding cytostatic agents in vitro. Dose-response curves with untreated (sensitive) and drug-pretreated (resistant) tumor lines of the leukemia L1210 in the short-term test. *Abscissa*, different dose levels of cytostatic agents. *Ordinate*, incorporation of [^{3}H]uridine (doxorubicin, daunorubicin) or [^{3}H]thymidine (cytosine arabinoside, mafosphamide) into the cells (percentage of control values). Each point represents the average from two measurements. ▲-▲, Sensitive parental tumor line, △···△, drug-pretreated tumor line

fold for $L1210_{dox}$ cells, for $L1210_{dnr}$ cells 30-fold, for $L1210_{AraC}$ cells 2500-fold, and 3-fold for $L1210_{ctx}$ (Table 1). We found a multidrug resistance only in the $L1210_{dox}$ and $L1210_{dnr}$ tumor lines, which means that there exists a cross-resistance between doxorubicin, daunorubicin and actinomycin D, but no resistance to

Table 1. Degree of resistance and cross-resistance of tumor lines to different cytostatic drugs determined by the short-term test. Mean values of four measurements

Drug	$L1210_{dox}$	$L1210_{dnr}$	$L1210_{AraC}$	$L1210_{ctx}$
Doxorubicin	45	51	0.4	0.5
Daunorubicin	24	30	0.8	0.8
Actinomycin D	12	20	0.2	0.8
Cytosine arabinoside	0.9	0.3	2500	2
Mafophosphamide[a] (Asta-Z-7557)	–	0.3	0.5	3

[a] A derivative of cyclophosphamide, which is active in vitro

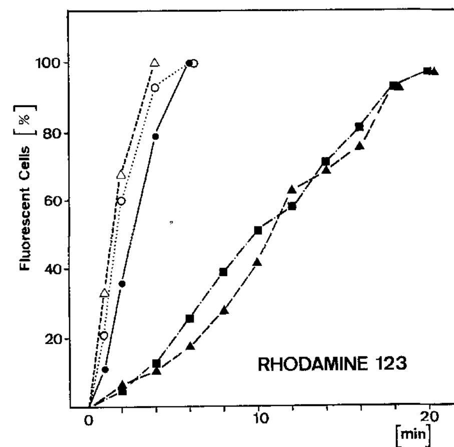

Fig. 3. Detection of multidrug resistance in the different tumor lines by the accumulation assay using rhodamine-123 (5 μg/ml). *Abscissa*, time after addition of rhodamine-123. *Ordinate*, percentage of fluorescent cells. ●-●, Parental tumor line L1210; ▲--▲, $L1210_{dox}$; ■--■, $L1210_{dnr}$; ○···○, $L1210_{AraC}$; △--△, $L1210_{ctx}$. Each point represents the median of ascites tumor cells from three animals (three measurements per animal)

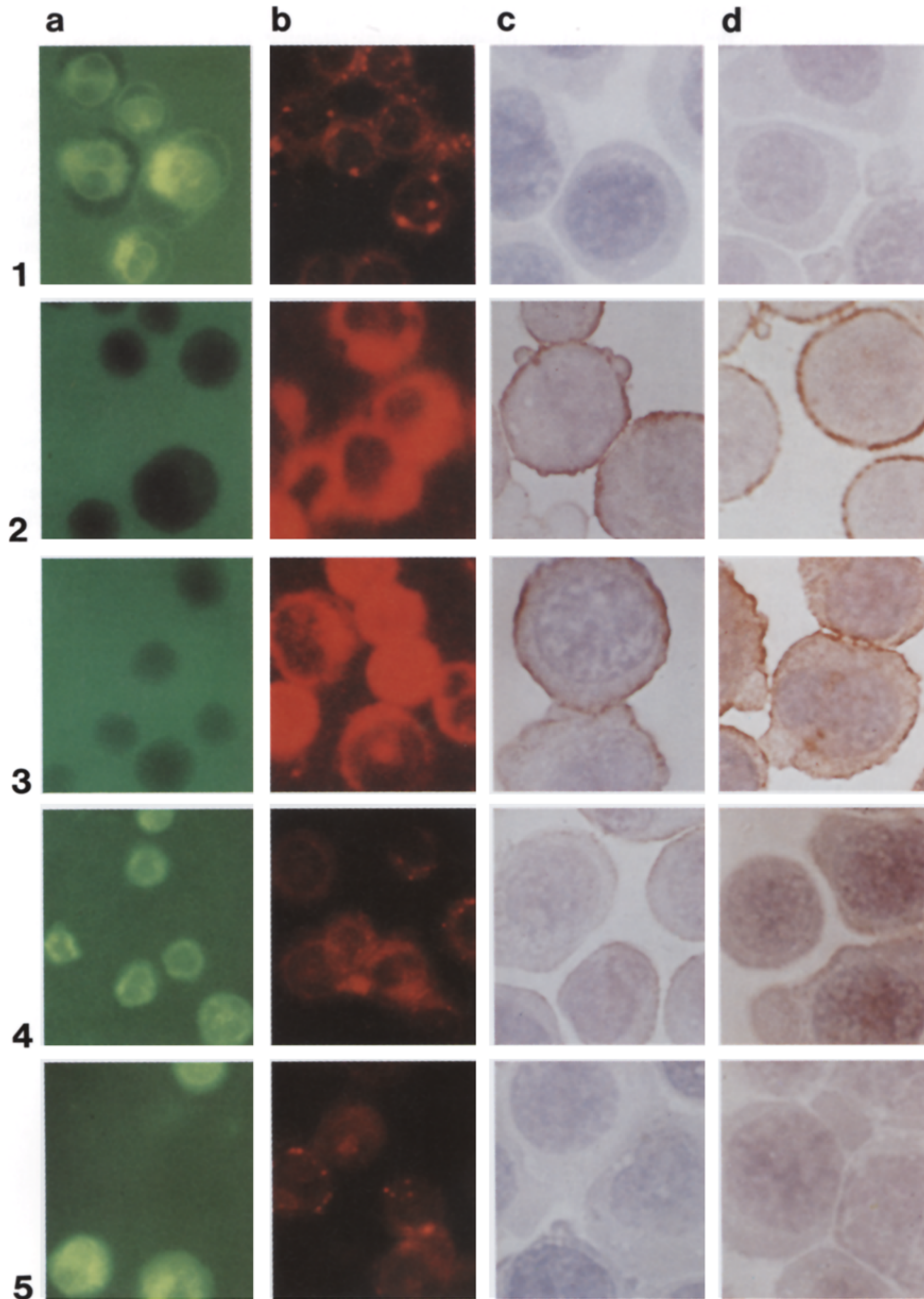

Fig. 4. Detection of multidrug resistance in the different tumor lines (1–5) using several morphological methods (a–d): (a) accumulation of rhodamine-123 (5 µg/ml) after incubation for 5 min (×500); (b) immunofluorescence with the streptavidin/biotin/phycoerythrin method using monoclonal antibody (mAb) 265/F4 (×500); (c) immunoperoxidase staining with mAb 265/F4 (×1200); (d) Immunoperoxidase staining with mAb C219 (×1200). Line 1, sensitive parental L1210 cells; line 2, $L1210_{dox}$ cells; line 3, $L1210_{dnr}$ cells; line 4, $L1210_{AraC}$ cells; line 5, $L1210_{ctx}$ cells

cytosine arabinoside and mafophosphamide. This MDR phenotype does not exist in the $L1210_{AraC}$ and $L1210_{ctx}$ tumor lines.

Detection of multidrug resistance by the rhodamine-123 accumulation assay

Cross-resistance of rhodamine-123 (R-123) to the anthracyclines has been described (Tapiero et al. 1984). Therefore, we determined the R-123 accumulation in the sensitive and resistant tumor lines. We found that $L1210_{dox}$ and $L1210_{dnr}$ cells need more time to accumulate R-123 than their sensitive parental cells (Fig. 3). In contrast to these data, the $L1210_{AraC}$ and the $L1210_{ctx}$ need less time. In Fig. 4 sensitive L1210 cells (a1) and the non-multidrug-resistant cell lines $L1210_{AraC}$ (a4) and $L1210_{ctx}$ (a5) show a significant rhodamine fluorescence, whereas the $L1210_{dox}$ cells (a2) and $L1210_{dnr}$ cells (a3) do not. Thus, with this accumulation assay we can detect the multidrug resistance.

Expression of P170 glycoprotein

In order to find out whether only resistant tumor cells of the MDR phenotype show alterations in the plasma membranes we compared resistant cells that express the MDR phenotype with those that do not express this resistance type, using the monoclonal antibodies 265/F4 and C219. These antibodies were prepared against the membrane glycoprotein P170 in colchicine-resistant CHO cells. Figure 4 demonstrates that $L1210_{dox}$ (b2) and $L1210_{dnr}$ (b3) tumor cells show an intense immunofluorescence reaction using the monoclonal antibody 265/F4. In contrast, no specific immunostaining was observed in parental (sensitive) (b1) and $L1210_{AraC}$ (b4) or $L1210_{ctx}$ (b5) tumor cells. Similar results were obtained by light microscopy using the streptavidin/biotin/horseradish-peroxidase method and the monoclonal antibodies 265/F4 (column c) and C219 (column d). The sensitive (c1,d1) or $L1210_{AraC}$ (c4,d4) and $L1210_{ctx}$ cells (c5,d5) reveal in general no or only a weak staining in a few cells, whereas $L1210_{dox}$ (c2,d2) and $L120_{dnr}$ cells (c3,d3) show an intense membrane-bound staining. These results could be confirmed by immunoblotting (Fig. 5). $L1210_{dox}$ and $L120_{dnr}$ tumor cells displayed elevated levels of the 170-kDa membrane glycoprotein. No or less expression of this protein could be observed in sensitive or non-multidrug-resistant tumor lines. Thus, in this study we found an increased expression of the protein of 170 kDa in $L1210_{dox}$ and $L1210_{dnr}$ tumor lines, while the expression of this protein was weak in sensitive and in $L1210_{AraC}$ or $L1210_{ctx}$ tumor lines.

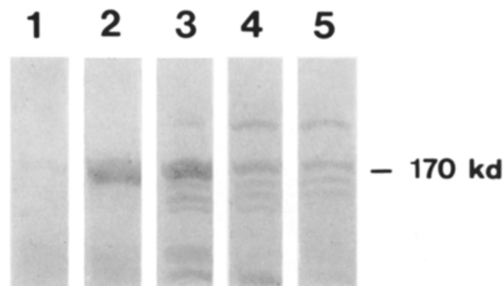

Fig. 5. Western blot analyses. Plasma membranes were prepared, SDS-PAGE was performed and gels were blotted onto nitrocellulose paper and probed with antibody 265/F4. 10 μg protein was loaded per lane. 1, Parental (sensitive) L1210; 2, $L1210_{dox}$; 3, $L1210_{dnr}$; 4, $L1210_{AraC}$; 5, $L1210_{ctx}$

Expression of multidrug-resistant DNA sequences in resistant cells

To determine whether multidrug-resistant DNA-sequences were expressed in the resistant cells which express the MDR phenotype, Northern blots were performed with RNA of sensitive and resistant cells (Fig. 6). Using the clone pcDR1.5, elevated RNA levels were detected in $L1210_{dox}$ and $L1210_{dnr}$ tumor cells. Less expression could be observed in sensitive and non-multidrug-resistant tumor lines. The lack of difference in α-actin gene expression in the sensitive and resistant cell lines suggests that comparable levels of RNA were loaded on the gels (data not shown).

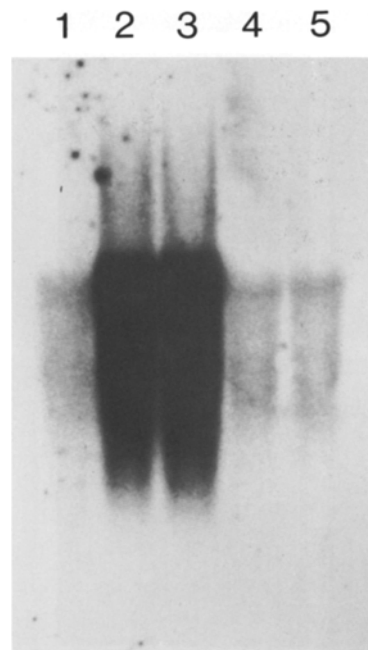

Fig. 6. Northern blot analyses. Hybridization with the labeled cDNA probe pcDR1.5 (10 μg RNA). 1, Parental (sensitive) L1210 cells; 2, $L1210_{dox}$ cells; 3, $L1210_{dnr}$ cells; 4, $L1210_{AraC}$ cells; 5, $L1210_{ctx}$ cells

Discussion

Many laboratories have attempted to develop test systems to characterize the sensitivity or resistance of tumors to drugs (for review see Mattern and Volm 1982). Although some procedures have achieved clinical importance in a few centers, no one test system has acquired widespread clinical acceptance and use. It is, therefore, not surprising that a general disillusionment has developed in this area of research.

In the last few years the concept of pleiotropic or multidrug resistance has been developed (Ling 1982). This cross-resistance is often associated with specific alterations in tumor cell membranes. High-molecular-mass compounds of natural product origin have been especially associated with the presence of a specific membrane glycoprotein marker (Juliano and Ling 1976; Beck et al. 1979; Giavazzi et al. 1984; Kartner et al. 1985; Shen et al. 1986; Volm et al. 1987). The aim of this investigation, therefore, was to find out whether only resistant cells of the MDR phenotype can be recognized by the monoclonal antibodies or whether resistant cells that do not reveal the MDR phenotype can also be differentiated using the monoclonal antibodies 265/F4 and C219. We compared resistant cell lines that express the MDR phenotype ($L1210_{dox}$ and $L1210_{dnr}$) with those that do not ($L1210_{AraC}$ and $L1210_{ctx}$), by immunofluorescence, immunocytochemistry and immunoblotting. The results of this study demonstrate that only resistant cells with the MDR phenotype show an increased expression of the 170-kDa membrane glycoprotein and this was confirmed by estimation of the expression of DNA sequences associated with multidrug resistance. These results could be one of the reasons why in many resistant human tumors no changes in the membrane glycoprotein P170 could be detected (Bell et al. 1985; Gerlach et al. 1987). Bell and coworkers (1985) could detect the plasma membrane protein P170 only in two out of five patients with resistant ovarian carcinoma, using Western blot analysis. Gerlach et al. (1987) demonstrated that only 4 out of 46 tumor specimens of patients representing 12 tumor types displayed elevated levels of P-glycoprotein and that this mode of resistance may have some clinical significance only in sarcoma patients.

It remains to be clarified that this protein is really linked with the transport modifications and does not represent the consequence of another still unknown event. In this study, therefore, we determined the R-123 accumulation in the sensitive and resistant tumor lines. We found that the $L1210_{dox}$ and $L1210_{dnr}$ cells need more time to accumulate R-123 than the cells of their sensitive parental tumor line. In addition the $L1210_{AraC}$ and $L1210_{ctx}$ cells need less time than the $L1210_{dox}$ and $L1210_{dnr}$ cells. Thus, in this accumulation assay we could detect the multidrug resistance. These results seem to indicate that these changes in the plasma membranes are really linked with transport modifications.

Although overexpression of P-glycoprotein may not be the only mechanism for multidrug resistance, according to these data the plasma membrane glycoprotein P170 could eventually become both a prognostic tool to indicate resistant human tumor cells with the MDR phenotype and a target molecule for chemotherapy to eliminate resistant cells of this resistance type. But this will be true only for a small group of resistant tumors. In addition, before such approaches are possible, a greater knowledge of the distribution and function of this membrane protein in different tumors is required. Therefore, investigations on human tumors are continuing in our laboratory.

Acknowledgements. We wish to thank Dr. B. Lathan and Dr. V. Ling for the generous provision of the antibodies, and Dr. J. Croop for the probe pcDR1.5.

References

Bak M, Mattern J, Volm M (1987) Maintenance of morphology and tumour marker production in human epidermoid lung carcinoma xenografts. In vivo 1:319–326

Beck WT, Müller TJ, Tanzer LR (1979) Altered surface membrane glycoproteins in vinca alkaloid resistant human leukemia lymphoblasts. Cancer Res 39:2070–2076

Bell DR, Gerlach JH, Kartner N, Buick RN, Ling V (1985) Detection of P-glycoprotein in ovarian cancer: a molecular marker associated with multidrug resistance. J Clin Oncol 3:311–315

Biedler JL, Chang T, Meyers MB, Peterson RHF, Sprengler BA (1983) Drug resistance in Chinese hamster lung and mouse tumor cells. Cancer Treat Rep 67:859–867

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Danø K (1973) Active outward transport of daunomycin in resistant Ehrlich ascites tumor cells. Biochem Biophys Acta 323:466–483

Fairbanks G, Steck TL, Wallach DFH (1971) Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10:2606–2617

Gerlach JH, Bell DR, Karakousis G, Slocum HK, Kartner N, Rustum YM, Ling V, Baker RM (1987) Glycoprotein in human sarcoma: evidence for multidrug resistance. J Clin Oncol 5:1452–1460

Giavazzi R, Kartner N, Hart IR (1984) Expression of cell surface P-glycoprotein by an adriamycin-resistant murine fibrosarcoma. Cancer Chemother Pharmacol 13:145–147

Gros P, Croop J, Roninson I, Varshavsky A, Housman DE (1986) Isolation and characterization of DNA sequences amplified in multidrug-resistant hamster cells. Proc Natl Acad Sci USA 83:337–341

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Juliano RL, Ling V (1976) A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochem Biophys Acta 455:152–162

Kartner N, Evernden-Porelle D, Bradley G, Ling V (1985) Detection of P-glycoprotein in multidrug-resistant cell lines by monoclonal antibodies. Nature 316:820–823

Lathan B, Edwards DP, Dressler LG, Von Hoff DD, McGuire WL (1985) Immunological detection of chinese hamster ovary cells expressing a multidrug resistance phenotype. Cancer Res 45:5064–5069

Ling V (1982) Genetic basis of drug resistance in mammalian cells. In: Bruchovsky N, Goldie JH (eds) Drug and hormone resistance in neoplasma, vol 1. CRC Press, Miami, pp 1–19

Mattern J, Volm M (1982) Clinical relevance of predictive test for cancer chemotherapy. Cancer Treat Rev 9:267–298

Riordan JR, Ling V (1979) Purification of P-glycoprotein from plasma membrane vesicles of Chinese hamster ovary cell mutants with reduced colchicine permeability. J Biol Chem 254:12701–12705

Riordan JR, Ling V (1986) Genetic and biochemical characterization of multidrug resistance. Pharmacol Ther 28:51–75

Riordan JR, Denchars K, Kartner N, Alon N, Trent J, Ling V (1985) Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines. Nature 316:817–819

Roninson IB, Abelson HT, Housman DE, Howell N, Varshavsky A (1984) Ampliciation of specific DNA sequences correlates with multidrug resistance in Chinese hamster cells. Nature 309:626–628

Shen D, Cardarelli C, Hwang J, Cornwell M, Richert N, Ishii S, Pastan I, Gottesman MM (1986) Multiple drug-resistant human KB carcinoma cells independently delected for high-level resistance to colchicine, adriamycin, or vinblastine show changes in expression of specific proteins. J Biol Chem 261:7762

Skovsgaard T (1978) Carrier-mediated transport of daunorubicin, adriamycin and rubidazone in Ehrlich ascites tumor cells. Biochem Pharmacol 27:1221–1227

Tapiero H, Munck JN, Fourcade A, Lampidis TJ (1984) Cross-resistance to rhodamine 123 in adriamycin- and daunorubicin-resistant Friend leukemia cell variants. Cancer Res 44:5544–5549

Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354

Volm M, Brüggemann A, Günther M, Kleine W, Pfleiderer A, Vogt-SchadenM (1985) Prognostic relevance of ploidy, proliferation, and resistance predictive tests in ovarian carcinoma. Cancer Res 45:5180–5185

Volm M, Efferth TH, Günther A, Lathan B (1987) Detection of murine S 180 cells expressing a multidrug resistance phenotype using different in vitro test systems and a monoclonal antibody. Arzneim Forsch Drug Res 37:862–867

Volm M, Drings P, Hahn EW, Mattern J (1988) Prediction of the clinical chemotherapeutic response of stage III lung adenocarcinoma patients by an in vitro short term test. Br J Cancer 57:198–200

Received July 20, 1988/Accepted October 6, 1988