P-GLYCOPROTEIN EXPRESSION IN LEUKEMIA P-388 CELLS WITH INDUCED RESISTANCE TO DOXORUBICIN

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The appearance of multiple drug resistance (MDR) in tumors during a course of chemotherapy narrows the scope for the use of cytostatics. This phenomenon is known for several different tumor cells. It has been shown that MDR is connected with the development of the unique ability of the tumor cells to reject toxic substances due to the formation of new structures in the plasma membrane that perform the role of a pump [2]. It has been shown that MDR correlates with the accumulation of specific proteins, namely so-called P-glycoproteins (P-GP), in the plasma membranes.

Depending on the type of cells, the molecular weight of P-GP varies from 130 to 210,000, and most frequently it is of the order of 150-170,000 daltons [2]. However, cases are known when MDR was not connected with P-GP expression and amplification of the MDR gene [8]. The aim of this investigation was to determine the P-GP level in leukemia P-388 cells, sensitive to doxorubicin (P388/0) and with induced resistance to the antibiotic (P388/DX).

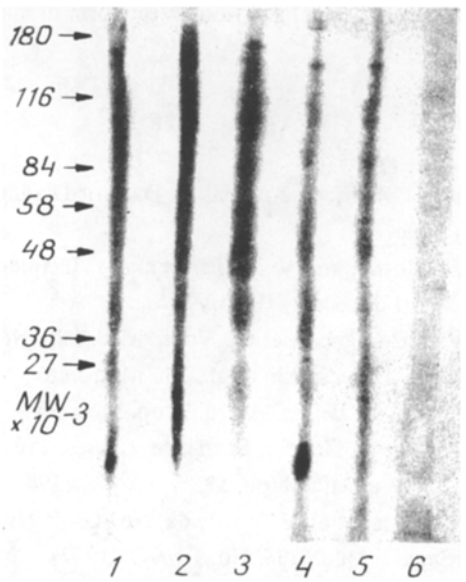

Fig. 1. Blot analysis of glycoprotein composition of resistant P388/DX cells (lanes 1-3) and P388/0 cells sensitive to doxorubicin (lanes 4-6). 1, 4) Homogenates, 2, 5) microsomal fractions, 3, 6) fractions of plasma membranes. Arrows and numbers on left indicate position of molecular weight markers (kilodaltons).

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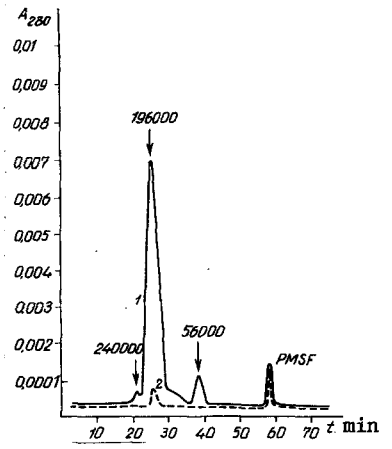

Fig. 2

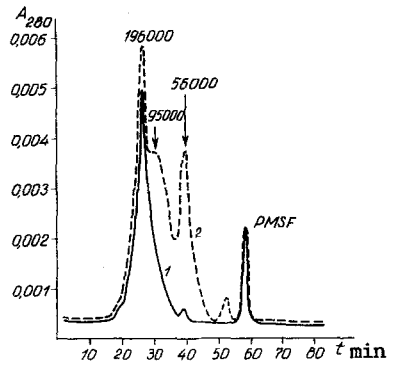

Fig. 3

Fig. 2. HPLC analysis of P-GP-RCA_1-specific fractions: 1) P388/DX cells, 2) P388/0 cells. Numbers above arrows indicate molecular weight of eluted proteins.

Fig. 3. Effect of Lubrol PX on resistance of P-GP. P-GP fractions treated (1) with 1% Lubrol PX and incubated at room temperature for 30 min (2). Numbers above arrows and peaks show molecular weight of eluted proteins; PMSF) phenylmethylsulfonyl fluoride,.

EXPERIMENTAL METHOD

Leukemia P388/0 and P388/DX cells were obtained as described previously [1]. Plasma membranes of the leukemia cells were isolated by centrifugation in a Percoll gradient as in [6]. About 10^9 cells were used to isolate the membranes.

P-GP were isolated from the plasma membranes by the method described for isolating P-GP from Chinese hamster ovarian cells [7], followed by concentration of the β-D-galactose-containing glycoprotein fractions by ultrafiltration. Fractions of P-GP were analyzed by HPLC on a TSK-G-3000-SW gel-filtration column, previously calibrated against proteins of different molecular weights. The eluting solution was 0.05 M Tris-HCl buffer, pH 7.4, containing 0.1 M NaCl and 0.1% sodium N-lauroyl-sarcosinate Electroblotting was carried out by a semidry method over a period of 2 h, with a current of 0.8 mA/cm gel. The nitrocellulose membrane was blocked with a solution containing 3% BSA in 0.01 M phosphate buffer, pH 7 2, for 1 h, and then in ricin for 1 h, then washed 3 times for 10 min in 0.01 M phosphate buffer, after which the membrane was stained by the Con A-peroxidase method, as described in [4]. Protein in the samples was determined by the method in [5].

EXPERIMENTAL RESULTS

Plasma membranes isolated from leukemia P388/0 and P388/DX cells were analyzed by electrophoresis and a lectin-enzyme method. The data in Fig. 1 show that the resistant cells (lanes 1-3) contain much more galactoproteins than sensitive cells (lanes 4-6). Under these circumstances, α-D-Glu and α-D-Mal residues may also appear to a very slight degree. The plasma membrane proteins were solubilized with buffer containing sodium N-lauroylsarcosinate or 1% Lubrol PX, or a mixture of detergents: 1% Lubrol PX, 1.5% Triton X-100, and 0.5% octyl-β-D-glucopyronoside. However, as was subsequently discovered, after affinity chromatography the Lubrol PX and Triton X-100, despite resulting in more complete solubilization of plasma membrane proteins, caused degradation of the P-GP to products with lower molecular weight and, for that reason, they cannot be recommended for use in isolating P-GP (Fig. 3).

The solubilized plasma membrane proteins were separated by affinity chromatography on RCA_1-sepharose 4B. The quantity of protein detected in P-GP fractions from P388/0 cells varied from 0 to 0.045 mg/10^9 cells, whereas for P388/DX cells it was 0.51 $\pm$ 0.58 mg/10^9 cells, whereas for P388/DX cells was more than an order of magnitude greater than in the sensitive P388/0 cells. In the plasma membranes of the P388/DX cells, P-GP accounted for 4-4.5% of the total protein.

Analysis of the P-GP fractions by HPLC showed that the resistant cells contained mainly P-GP with mol. wt. of 196,000, together with a small quantity of proteins with mol, wt. of 240,000 and 56,000 (Fig. 2). Analysis of RCA-specific proteins isolated from P388/0 cells, in which protein was detected, revealed a very small peak with mol. wt. of 196,000 (Fig. 2, curve 2). Attempts at further purification of the protein by gel-filtration from P388/DX cells were unsuccessful, for after collection of the P-GP fractions with mol. wt. of 196,000, their concentration by ultrafiltration and repeated gel-chromatography, a satellite protein with mol. wt. of 56,000 and an unidentified low-molecular-weight product "X," eluted almost simultaneously with PMSF, were formed de novo. The widened shoulder of the peak with mol. wt. of 196,000 in the high-molecular-weight region may be connected with partial aggregation of P-GP.

Thus the multiple drug resistance arising in leukemia P388/DX cells is characterized by a high P-5P level in the plasma membranes.

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