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Vinblastine sensitivity of leukaemic lymphoblasts modulated by serum lipid

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The high-resolution proton magnetic resonance spectrum of leukaemic lymphoblasts is characteristic of neutral lipid in an isotropic environment. When such lymphoblasts are selected for resistance to the anticancer drug vinblastine, the intensity of this spectrum increases with increasing drug resistance. A reversal of this trend can be achieved by growing cells in delipidated serum, whereby lipid spectrum and drug resistance are diminished. However, both can be restored by subsequent regrowth in normal medium. Thus, although detectable genetic changes accompany the development of vinblastine resistance, the expression of these changes can be modulated by environmental lipid.

Vinblastine; Drug resistance; NMR, ^{1}H-; Lipid content; (Leukemic lymphoblast)

1. INTRODUCTION

The development of cellular resistance to anticancer drug treatment is a major problem in cancer therapy which in some cases may be accompanied by the development of cross-resistance to chemically unrelated compounds [1,2]. There is now ample evidence that multidrug resistance arises from the amplification of the *mdr*1 gene, whose product is the plasma membrane P-glycoprotein which acts as an ATP-driven drug efflux pump [3–6]. However, other changes such as enhanced mitochondrial function [7,8] and alteration to the plasma membrane lipid composition [9,10] have been observed in multidrug-resistant cells. Furthermore, this type of multidrug resistance can be reversed in vitro by treatment with the calcium channel blocker verapamil [11,12], indicating that environmental factors can alter the expression of the cellular resistance.

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We have previously established that high-resolution ^{1}H NMR signals obtained from cancer cells arise from neutral lipid in the plasma membranes. A new membrane model has been proposed to account for the physical and chemical characteristics of the plasma membrane of malignant cells [13].

These NMR signals have been used to assess the metastatic status of a rat mammary adenocarcinoma model [14]. We have also shown that the intensity of the lipid signal increases with increasing drug resistance [15].

How such lipid domains may be involved in drug resistance is unknown, although vinblastine treatment has been shown to cause accumulation of very low density lipoprotein (VLDL) secretory vesicles in hepatocytes [16]. Our work has indicated that the drug itself interacts differently with the lipid domains in sensitive and resistant cells [15]. Moreover, lipoproteins have been used to transport drugs such as adriamycin more effectively into cells [17].

One method to test whether neutral lipid domains are important in drug resistance is to

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study the effects of reducing their concentration in cells. The lipid content of cultured cells can be modified by changes in their growth medium, in particular by depletion of serum lipoproteins. Here, we have grown a human acute lymphoblastic leukaemic T cell line resistant to vinblastine in medium containing delipidated serum and shown a reduction in the neutral lipid content of the cells and a concomitant decrease in the level of vinblastine resistance.

2. MATERIALS AND METHODS

2.1. *Cell lines and tissue cultures*

Cells of the acute lymphoblastic leukaemic T cell line CCRF-CEM, both sensitive and resistant to 5–10 ng/ml of vinblastine, were grown in RPMI-1640 medium supplemented with 10% foetal calf serum. The vinblastine-resistant cells were developed as reported [18]. The plasma membrane P-glycoprotein associated with vinblastine resistance is increased 3-fold in these cells [19]. All experiments were performed on cells which had been grown in drug-free medium for at least 2 weeks.

Delipidated foetal calf serum was prepared by a cold ether extraction method [20]. The protein was redissolved in a volume of water equivalent to the initial volume of serum and sterilized by filtration. Recovery of protein was quantitative but no lipid could be weighed on a Mettler MK60 balance after chloroform/methanol extraction of 5 ml serum.

In the delipidated serum experiments vinblastine-resistant cells were grown in RPMI-1640 supplemented with 10% delipidated serum (delipidated medium).

IC_{50} measurements were made as in [18]. The IC_{50} is defined as the concentration of drug that inhibits the 72 h growth of cell cultures by 50% when compared with the drug-free control cultures. Control cells doubled a minimum of twice during this period.

2.2. *Preparation of samples for NMR spectroscopy*

Cell samples were prepared for NMR experiments as described [15]. Cell viability was measured by dye exclusion before and after each experiment. Only those data obtained from cells with at least 90% viability were used.

2.3. *NMR spectroscopy*

^1H NMR spectra were recorded at 37°C using a Bruker AM 500 spectrometer. Peaks were referenced to aqueous sodium 3-(trimethyl-silyl)propanesulfonate. Two-dimensional correlated COSY spectra were recorded with a pulse sequence modified to compensate for rf inhomogeneity [21].

2.4. *Lipid extraction*

Cells (10^8) were pelleted at 700 × *g* for 10 min and washed three times with 45 ml physiological saline. The washed cells were resuspended in 2 ml of 10 mM Hepes (pH 7.2) and extracted as described in [22].

Total protein, lipid, triglyceride, phospholipid and free and esterified cholesterol were measured on the whole-cell extracts [22].

3. RESULTS

3.1. *Cellular resistance*

The resistant cell line grown in the absence of vinblastine for 2 weeks prior to experimentation had an IC_{50} of 16 ng/ml. In contrast, the sensitive parent line had an IC_{50} of 3 ng/ml which was constant over a 31-day period.

IC_{50} measurements (table 1) showed that the resistant cells became more sensitive to drug treatment during the 13 days of growth in delipidated medium. Although the IC_{50} varied between 10 and 12 ng/ml over the following 2 weeks of culture, there was an overall 38% mean loss of resistance.

Cell doubling times during culture in delipidated medium were variable (21–37 h). However, we conclude from the data that the IC_{50} is independent of growth rate.

When cells were returned to full medium, drug resistance was regained after 2 weeks in culture, without added vinblastine.

3.2. *^1H NMR spectroscopy*

The ^1H NMR spectra of the vinblastine-sensitive and -resistant CCRF-CEM cell lines are shown in fig.1A and B, respectively. As reported previously [15] the intensity of the lipid resonances increases with drug resistance. The COSY spectrum of resistant cells is shown in fig.2A. Assignments of lipid resonances are as in structure 1 and have been previously reported [21]. Cross-peaks Y and Y^1 have been assigned to fucosylated species on the plasma membrane [14].

The NMR spectrum of the resistant cell line changed dramatically after the cells were cultured in delipidated medium for 13 days (fig.1C). The

Table 1

Effect of growing vinblastine-resistant leukaemic lymphoblasts in delipidated medium

Days of growth	Doubling time (h)	IC_{50} (ng vinblastine per ml)	Loss of resistance (%)
0	22	16	
13	37	10 (16)	39
25	35	12 (19)	37
31	23	10 (16)	38

The IC_{50} is based on the 72 h growth of cell cultures as described in section 2. The IC_{50} of cells in full growth medium is given in parentheses

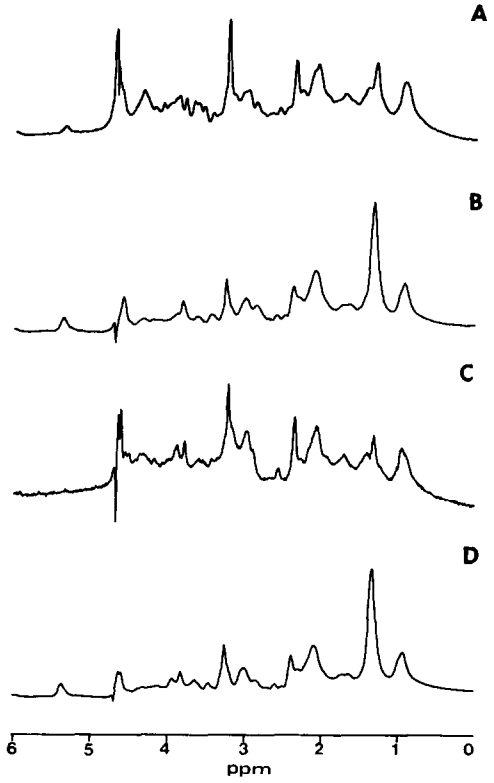


Fig.1. One-dimensional ^1H NMR spectra of: (A) CCRF-CEM cells (1×10^8) sensitive to vinblastine; (B) CCRF-CEM cells resistant to 10 ng/ml of vinblastine; (C) resistant cells grown in delipidated serum; (D) as for (C) but regrown in full medium. Spectra were recorded at 37°C with the sample spinning. Cells were suspended in PBS/D_2O and the residual HOD peak was suppressed by gated irradiation.

one-dimensional NMR spectrum was poorly resolved with a significant decrease in the intensity of the acyl chain resonances at 1.3 ppm (-CH_2-). This indicates a major reduction in the amount of isotropically tumbling neutral lipid.

The broadening of these signals is confirmed in the COSY spectra (fig.2B). None of the cross-peaks associated with triglyceride is apparent although cross-peaks arising from cellular metabolites are still present.

When the cells were grown again in full medium, they regained their high-resolution lipid signal (fig.1D) as well as their drug resistance.

3.3. *Lipid composition*

Total lipid, protein, free and esterified cholesterol, triglyceride and phospholipid levels in

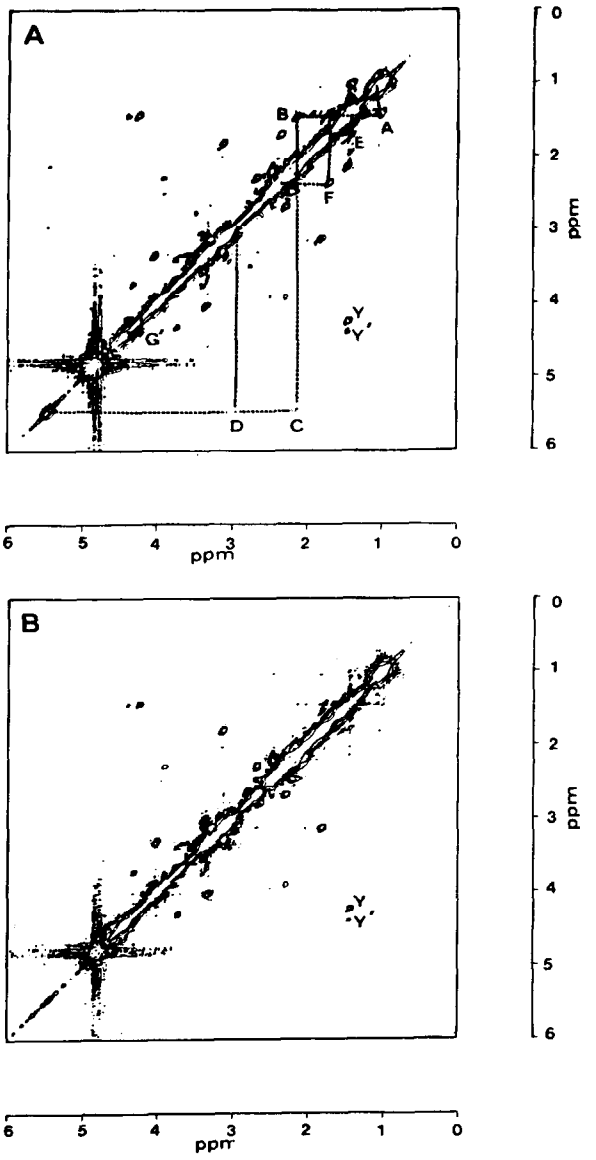
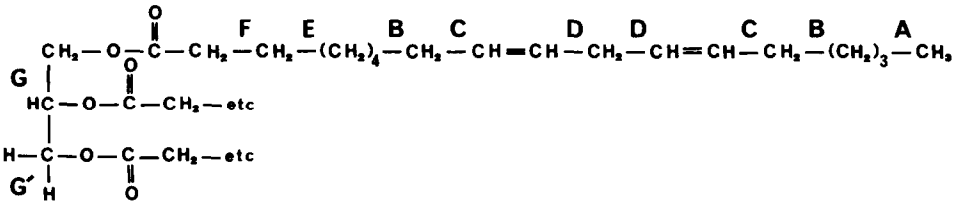


Fig.2. Two-dimensional symmetrised COSY spectra of: (A) CCRF-CEM cells (1×10^8) resistant to vinblastine; (B) resistant cells grown in delipidated serum. Sinebell and Gaussian (LB = −30, GB = 0.25) window function were applied in the t_1 and t_2 domains, respectively. Lipid acyl chain assignments are indicated according to structure 1. Cross-peaks Y and Y′ have been assigned to fucose and lactate, respectively.

resistant cell lines cultured in normal and delipidated medium are listed in table 2.

After culture of the resistant cell line in delipidated medium for 28 days, all four classes of lipids assayed were diminished 2–3-fold although



Structure 1

Table 2

Whole-cell lipid and protein composition of vinblastine-resistant cells grown in normal and delipidated medium

	Normal serum	Delipidated serum
Free cholesterol	245 ± 18	129 ± 54
Cholesterol ester	51 ± 9	22 ± 19
Triglyceride	70 ± 4	39 ± 15
Phospholipid	1035 ± 72	456 ± 121
Total lipid	1.37 ± 0.14	1.49 ± 0.80
Total protein	1.8 ± 0.1	2.20 ± 0.6

Data are expressed as nmol per 10^8 cells for the four lipid classes and the values represent the mean ± SD determined from duplicate assays on three different extractions. The total lipid and protein content are expressed as mg per 10^8 cells

total protein and lipid levels were maintained. Not all the lipid can be accounted for in the delipidated cells, but the neutral lipids which give rise to the NMR signal are clearly reduced. The cholesterol:phospholipid ratio was maintained at 0.25 ± 0.01 in cells whether grown in normal or delipidated media. The triglyceride:phospholipid and triglyceride:cholesterol ester ratios were also maintained when the resistant cells were grown in delipidated medium despite the decrease in each of the four lipid classes.

We have previously shown that cells resistant to higher levels of vinblastine (20 ng/ml) have an increased total lipid and protein content [23]. The lipid data presented here are in agreement with this latter observation, since the resistant cells which became more drug sensitive following growth in delipidated medium also incurred a net loss of the four classes of lipid assayed.

4. CONCLUSIONS

Growth of leukaemic T cells in delipidated medium caused the high-resolution NMR spectra, indicative of isotropically tumbling lipid domains, to diminish. These neutral lipid domains have a lipid content which is largely triglyceride, and thus the NMR spectra indicate a decrease in cellular triglyceride or a change in the lipid's physical environment. These changes were accompanied by a 40% increase in sensitivity to vinblastine.

This effect, however, is reversible and after regrowth in full medium, the cells regained both their high-resolution NMR lipid signal and drug resistance.

It is clear that genetic factors, in particular amplification of the gene that codes for the P-glycoprotein, play a major role in cellular drug resistance [6]. However, the present data indicate that environmental conditions, in particular lipids in the medium, can modulate low levels of cellular resistance to vinblastine. We postulate that the NMR-sensitive lipid domains within the plasma membrane dissolve lipophilic drugs and thereby reduce their cellular toxicity. In the absence of serum lipid, cellular metabolism is altered such that these lipid domains are diminished or changed in structure and are no longer effective reservoirs for drugs. The documented changes in plasma membrane, cytoskeleton and mitochondria may be associated with the effectiveness of the transport of these lipid domains, and hence drug, out of the cells.

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