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Reversal of multidrug resistance and increase in plasma membrane fluidity in CHO cells with R-verapamil and bile salts

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Abstract

Studies with multidrug resistance modifiers indicate that perturbations of the cell membrane structure may influence P-glycoprotein (P-gp)-mediated drug transport. We describe studies of plasma membrane order using electron-paramagnetic resonance (EPR) in resistant (CH^RC5) and sensitive (AUXB1) chinese hamster ovary cells treated with R-verapamil and bile salts. Cell growth rates were determined in presence of doxorubicin mitomycin and cisplatin. The plasma membrane order in untreated resistant cells was higher than in the sensitive cells. Both the bile salt taurochenodeoxycholate (TCDC; 0.2–1.6 mM) and R-verapamil (1–3 μM) lowered the membrane order in the CH^RC5 cells to that in the sensitive cells and reversed the resistance to doxorubicin and mitomycin. The bile salt tauroursodeoxycholate (TUDC; 0.2–3 mM) did not lower membrane order and did not sensitise CH^RC5 cells. Neither R-verapamil, TCDC nor TUDC reduced the membrane order of the sensitive cells AUXB1 cells. These results support the view that changes in multidrug resistance in Chinese hamster ovary cells and P-gp function are associated with alterations in the fluidity of the plasma membrane.

Keywords: Multidrug-resistance; Cell membrane fluidity; Chinese hamster ovary cells; R-verapamil; Bile salts

1. Introduction

An important resistance mechanism in tumours is the expression of the *mdr-1* gene product, P-glycoprotein (P-gp), a 170 kd protein present in the plasma membrane. When this protein is highly expressed, the intracellular concentration of some cytostatic agents, e.g. anthracyclines and antitumor antibiotics, is reduced and it has been suggested that P-gp functions as a low specificity efflux pump for drugs and/or ions [1]. How P-gp functions as an active efflux pump or direct transporter for cytostatic drugs is not well understood, mainly because of the absence of specific structural requirements for molecules undergoing transport and for those inhibiting this process, the so-called resistance modifiers the classical example of which is the calcium antagonist verapamil [2,3]. The intracellular drug concentration, however, is dependent on both active and passive movement of the drug across the plasma membrane and we have therefore investigated the physical properties of the plasma membrane in P-gp-containing Chinese hamster ovary cells (CHO). The membrane potential $\Delta\psi$, pH-gradients (ΔpH), and the composition of membrane lipids can affect drug diffusion rates [4–6]. It is also known that differences in the chemical composition of membrane lipids exist between sensitive and resistant cells and that the structural order of the lipid membrane in CHO cells is higher in the resistant cell line [7,8]. Fluorescence anisotropy studies with the fluorescent probe 1-[4-trimethylammonium-phenyl]-6-phenyl-1,3,5-

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hexatriene (TMA-DPH) show a reduced mobility of the acyl chains in resistant CHO (CH^RC5) cell membranes compared with parental cells [9]. When CH^RC5 cells were grown in media rich in the C-17 saturated fatty acid, heptadecanoic acid, they accumulated vinblastine to the same extent as the parent cell line and CH^RC5 cells treated with verapamil. The same effect could not be achieved using the rigidifying fatty acid, stearic acid [10]. Although these authors did not obtain electron paramagnetic resonance measurements of membrane order to support their hypothesis, they concluded that perturbation of the membrane can reduce multidrug resistance. This conclusion is in agreement with an earlier report from this laboratory showing that physiological concentrations of R-verapamil increase membrane fluidity in these cells [11].

Several studies have shown that membrane fluidity changes may lead to increased accumulation of hydrophobic chromophores and antitumor agents [3,5,6,12,13]. These include studies with sensitising agents such as R-verapamil, reserpine and detergents and a variety of cell types including cells with high P-gp levels and artificial lipid bilayer models. However, none of these studies has been able to show where these changes take place or show a clear relationship between changes in plasma membrane order and reversal of multidrug resistance. This may in part be due to the use of conventional physicochemical methods of measurement such as absorption, light dispersion, viscosity, optical rotation and circular dichroism which record the sum of all structural changes affecting the whole molecule.

In the studies reported here, we have used electron paramagnetic resonance spectroscopy (EPR) which, in contrast to the methods mentioned above, provides specific structural data on macromolecules such as membrane phospholipids irrespective of whether the biological system being investigated is cloudy or opaque. We have recently used EPR to demonstrate membrane structure changes accompanying reversal of drug resistance in a human bladder carcinoma cell line [14]. We now report similar studies carried out with a Chinese hamster ovary resistant subline, CH^RC5, and parental AUXB1 cells *in vitro* in which we have produced perturbations of the plasma membrane using two bile salt probes, taurochenodeoxycholate (TCDC) and tauroursodeoxycholate (TUDC). These salts occur naturally in the body and have widely different physical properties. In a physiological milieu, TCDC is dimeric and planar (sandwich-like), hydrophobic, releases phospholipids and causes fluidisation of apolar regions where it accumulates. TUDC on the other hand forms chains, is hydrophilic, accumulates at the interface and in the apolar region of the lipid bilayer and stabilises lipoprotein membranes [15–19]. EPR was used to measure alterations in membrane order induced by these two probes. The biological significance of the property being measured is that it is an index of the changes induced at the polar/apolar interface, which is the main energetic border of the membrane and a determinant of the barrier properties of the membrane [20]. Parallel cell survival experiments were carried out using doxorubicin, mitomycin and cisplatin. The objective of the investigation was to examine the correlation between P-gp function in multidrug resistance and the fluidity and membrane order of the plasma membrane.

2. Materials and methods

2.1. Cells and culture conditions

The Chinese hamster ovary subline, CH^RC5, selected for resistance to colchicine [21] and the parental cell line (AUXB 1) were generously donated by M. Volm, German Cancer Research Center, Heidelberg, Germany. Cells were maintained as monolayers in 75 cm^2 flasks (Nunclon) at 37°C with 5% CO_2 and 100% humidity and propagated in α-Modified Eagle Medium (MEM) containing deoxyribonucleosides and ribonucleosides and supplemented with 10% fetal calf serum (FCS), 1% penicillin-streptomycin (5000 IU/ml) and 1% L-glutamine (200 mM; Gibco, Grand Island, NY). CH^RC5 cells were maintained in the presence of colchicine, 5 μg/ml (Sigma Chemical Co, Germany), but prior to experiments they were grown in the absence of colchicine for two transfers (7 days). Cell monolayers were harvested at 80–90% confluency and transferred to fresh medium every 3 days in order to sustain exponential growth. Initial cell density was 2×10^6 cells per 20 ml α-MEM-medium and $15–16\times10^6$ cells per flask with 20 ml medium after 3 days.

2.2. Determination of mdr-1-expression in CH^RC5 cells

Mdr-1-expression in CH^RC5 cells was determined using the reverse transcriptase-polymerase chain reaction (RT-PCR) following isolation of RNA and incubation with primer oligo$(dT)_{12–18}$. The PCR was carried out using the *mdr-1* primers CCC ATC ATT GCA ATA GCA GG (residues 2596–2615) and GTT CAA ACT TCT GCT CCT GA (residues 2733–2752). The *mdr-1*-specific PCR-product (167 base pairs) was separated on a 12% polyacrylamide gel, the bands stained with ethidium bromide and the fluorescence density quantitated photometrically.

2.3. Determination of cell proliferation rates

Cell suspensions containing 1000 cells were seeded into microwell culture plates and R-verapamil, TCDC and TUDC added at concentrations in the range 0.01–20 μM, 0.01–2 mM, 0.01–5 mM, respectively. The con-

centration of the cytostatic drugs were in the range 0.1–1.5 μg/ml (0.2–2.5 μM) for doxorubicin, 0.01–1.5 μg/ml (0.03–4.5 μM) for mitomycin and 0.05–2.5 μg/ml (0.2–8 μM) for cisplatin. Cells were incubated at 37°C in 5% CO_2 and 100% humidity for 1 to 5 days. Cell viability was determined according to the method of Larsson and Nygren [22] using fluorescein diacetate (Molecular Probes Inc., Eugene, OR, USA) prepared as a stock solution of 1 mg/ml dimethylsulphoxide and diluted with serum-free culture medium to a working concentration of 1 μg/ml. The fluorochrome fluorescein was quantitated as FU (fluorescence units) at 590 nm in a fluorescence microplate scanner (CytoFluor™ 2300; Millipore) using an excitation wavelength of 485 nm. Blank corrected means were based on six individual estimations and plotted as mean ±standard deviation (S.D.).

2.4. Cell cultures and incubations for EPR measurements

CH^RC5 cells and AUXB1 cells were harvested on the third day of growth by washing and suspending in FCS-free α-MEM-medium, centrifuging at 1000 *g* for 10 min, resuspending the pellet in 1 ml FCS-free medium and diluting in Eppendorf tubes to give 4×10^6 cells in 2 ml FCS-free medium. On the third day of growth, regarded as optimal for EPR-determinations [23], the viability of the cells, determined using methylene blue exclusion, was 85–95%. Incubations with 4×10^6 cells in 2 ml FCS-free medium were carried out with R-verapamil (Knoll AG, Ludwigshafen, Germany; stock solutions prepared in 0.9% NaCl) and the bile salts, TCDC and TUDC (Calbiochem, San Diego, CA; stock solutions prepared in FCS-free α-MEM medium) at 37°C using the following conditions: R-verapamil (0.01, 0.03, 0.1, 0.3, 1, 3, 10 μM) for 10 min, R-verapamil (0.3, 1, 3, 10 μM) for 60 min; TCDC (0.2, 0.5, 1, 1.6 mM) for 15 min, TCDC (0.1, 0.2 mM) for 120 min; TUDC (0.2, 0.5, 1, 1.6, 3.5 mM) for 15 min and TT.TUDC (0.1, 0.2 mM) for 120 min. The tubes were then centrifuged immediately (Eppendorf 3200, 10000 *g*, 2 min) and the pellet resuspended in 2 ml Dulbecco's PBS buffer (Gibco, Grand Island, NY) and recentrifuged. Cells were then resuspended in 60 μl PBS and mixed vigorously for 10 s with 1 μl of spin label stock solution (5 mM 5-doxyl-stearic acid in 100% ethyl alcohol). Unbound label was removed by washing with 1 ml buffer and centrifuging as above. The pellet was then resuspended in 60 μl buffer, transferred to a 50 μl capillary tube and the spectra determined immediately.

2.5. EPR spectroscopy

Measurements were made on whole cells at room temperature in a Bruker B-R 70 spectrometer with a B-B 25 magnet (Bruker, Karlsruhe, Germany) operating in the x-band which was linear and accurate to ±0.1 G. Instrument settings were: microwave frequency, 9.49 GHz; centre field, 3380 G; sweep width, 100 G; microwave power, 20 milliwatts; scanning time, 400 s; time constant, 1 sec; modulation amplitude, 1 G. Standard EPR procedures were used [24,25] in which the cell membrane order parameter 's' is derived from EPR spectra of the spin label 5-doxyl-stearic acid (Aldrich, Steinheim, Germany) using the formula of Gaffney and Lin [26]. Because the nitroxide group of the reporter molecule is situated within four carbon atoms of the polar carboxyl group, 5-doxyl-stearic acid gives information of mainly the polar part of the bilayer and the polar-/apolar-region interface [23]. When $s=0$, molecular mobility is unhindered and fluidity is maximal. When $s=1$ molecular motion is negligible (i.e. 'rigid glass' spectra are obtained [25]). The significance of the term membrane order is therefore as follows: In the biological membrane we observe a gradient [23] which stretches from a highly ordered zone in the polar-apolar interface region, where the order reaches values around 0.6–0.8, to the apolar membrane core where the order is approximately 0.2. Since order is a term reciprocal to molecular motion, we also obtain a reciprocal relationship between the order parameter, 's' which we have measured in this study, and the membrane fluidity [27].

2.6. Statistical analyses

Statistical comparisons of sample means were made using the unpaired Student's *t*-test. In all cases, the 0.05 level was considered to be statistically significant. Where appropriate, data in the text and table are expressed as mean±S.D.

3. Results

3.1. RT-PCR analysis

A *mdr-1*-specific PCR product (167 base pairs) was detected as an intense band in the polyacrylamide gel using the resistant CH^RC5 cells. This band was completely absent using AUXB 1 cells. These results correlated with the immunohistochemical detection of P-gp using anti-P-gp antibodies JSB-1 and C-219, where AUXB1 cells were P-gp-negative and CH^RC5 cells strongly positive with both monoclonal antibodies. The RT-PCR results also correlated with measurements of the IC50 inhibitory concentrations for the P-gp substrates doxorubicin and mitomycin, but not with cisplatin (AUXB1: doxorubicin 0.1 μg/ml, mitomycin 0.5 μg/ml; cisplatin 1.5 μg/ml. CH^RC5: doxorubicin 4.5 μg/ml, mitomycin 7.5 μg/ml; cisplatin 3.5 μ/ml).

3.2. In vitro *cytotoxicity assay*

Doxorubicin (Fig. 1), mitomycin (Fig. 2), and cisplatin (Fig. 3), at concentrations highly cytotoxic to AUXB 1 cells in *in vitro* cytotoxicity tests, had little or no effect on the proliferation rate of the resistant CH^RC5 cell-line but, when combined with R-verapamil (3 cdmM), doxorubicin (Fig. 1a) and mitomycin (Fig. 2a) were cytotoxic. As expected, R-verapamil did not sensitise CH^RC5 cell to cisplatin, being only effective for agents undergoing adenosine triphosphate (ATP)-mediated efflux (Fig. 3a).

The sensitising effect of R-verapamil was mimicked by TCDC (1 mM) with doxorubicin (Fig. 1b) and mitomycin (Fig. 2b) and, like R-verapamil, TCDC did not sensitise CH^RC5 cells to cisplatin (Fig. 3b). On the other hand, TUDC did not sensitise CH^RC5 cells to doxorubicin (Fig. 1c), mitomycin (Fig. 2c) or cisplatin (Fig. 3c) even when added in a concentration 3-fold higher (3 mM) than that for TCDC. When added alone to the incubation at the concentrations used above, R-verapamil, TCDC and TUDC had no effect on the proliferation rate of CH^RC5 cells (Figs. 1–3).

3.3. *EPR-measurements*

Not only do TCDC and TUDC differ in their ability to sensitise CH^RC5 cells to doxorubicin and mitomycin, they also differ in their effects on the structural order of the plasma membrane, '*s*' where $s=0$ at maximum fluidity and $s=1$ at maximum rigidity (Table 1). Data are presented as a table to enable the effects of TCDC and TUDC at equal concentration and incubation time to be compared directly and quantitatively.

The order '*s*' of the CH^RC5 subline (0.723 ± 0.003; mean±S.D.) was significantly higher than in the sensitive parental line AUXB1 (0.698 ± 0.003; mean±S.D.; $P< 0.001$) demonstrating an inherently higher structural rigidity in CH^RC5 cells. At concentrations equal or similar to those used in proliferation experiments, R-verapamil and the bile salt TCDC, but not TUDC

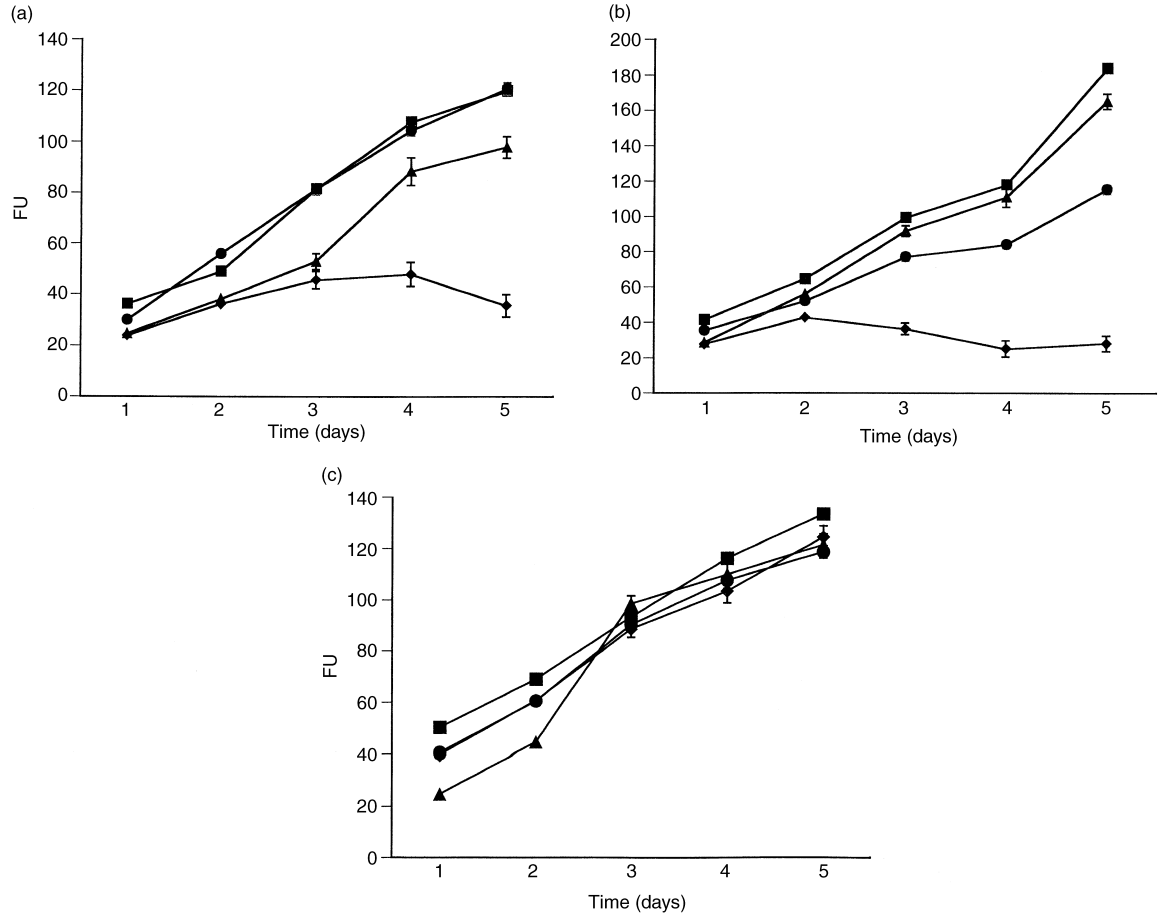

Fig. 1. Cell proliferation curves over 5 days for the CH^RC5 cell-line in the presence of doxorubicin (1 μg/ml) expressed as fluorescence units (FU): (a) effect of R-verapamil (3 μM): (■) control, (●) doxorubicin, (▲) 3 μM R-verapamil, (◆) combination of doxorubicin and 3 μM R-verapamil; (b) effect of TCDC (1 mM): (■) control, (●) doxorubicin, (▲) 1 mM TCDC, (◆) combination of doxorubicin and 1 mM TCDC; (c) effect of TUDC (3 mM): (■) control, (●) doxorubicin, (▲) TUDC, (◆) combination of doxorubicin and 3 mM TUDC.

reversed the higher plasma rigidity of CH^RC5 cells and abolished the difference in the order parameter between AUXB1 and CH^RC5 cells (Table 1). ED_{50} values for TCDC were 1 and 0.2 mM for 15 and 120 min, respectively, and for R-verapamil 0.03 μM at 10 min. Neither R-verapamil, TCDC nor TDUC reduced the membrane order of the sensitive AUXB1 cells. At the concentrations used, R-verapamil, TCDC and TUDC had no

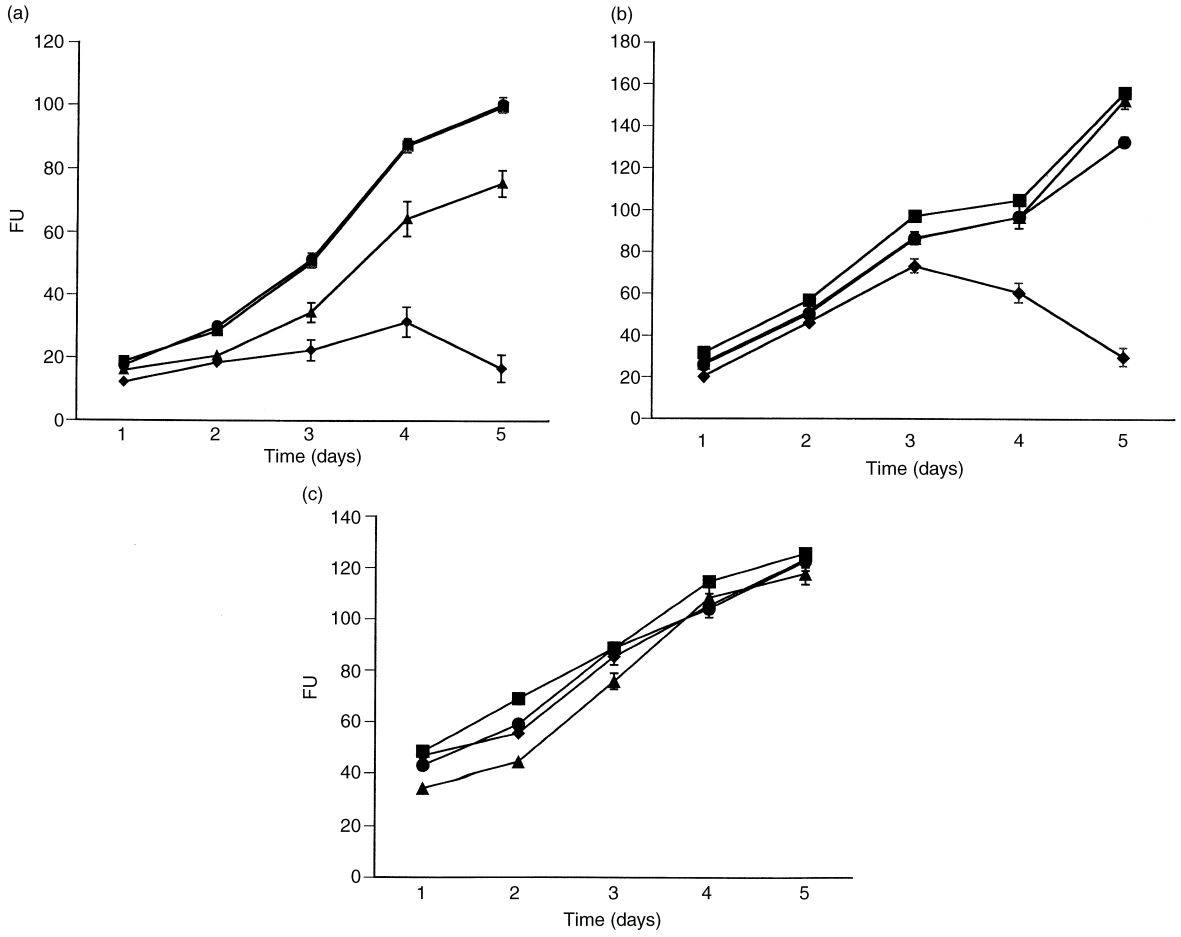

Fig. 2. Cell proliferation curves over 5 days for the CH^RC5 cell-line in the presence of mitomycin (1 μg/ml) expressed as fluorescence units (FU): (a) effect of R-verapamil (3 μM): (■) control, (●) mitomycin, (▲) 3 μM R-verapamil, (◆) combination of mitomycin and 3 μM R-verapamil; (b) effect of TCDC (1 mM): (■) control, (●) mitomycin, (▲) 1 mM TCDC, (◆) combination of mitomycin and 1 mM TCDC; (c) effect of TUDC (3 mM): (■) control, (●) mitomycin; (▲) TUDC, (◆) combination of mitomycin and 3 mM TUDC.

Table 1
Effect of R-verapamil, taurochenodeoxycholate (TCDC) and tauroursodeoxycholate (TUDC) on the plasma membrane order '*s*', of CH^RC5 cells[a]

Modulator	Membrane order parameter (*s*)	*P* value[b]	Percentage reversal of the rigidity (%)
None (control)	0.723±0.003		
R-Verapamil (1 μM/60 min)	0.696±0.008	<0.001	100
R-Verapamil (3 μM/10 min)	0.702±0.004	<0.001	84
TCDC (0.2 mM/120 min)	0.707±0.002	<0.001	64
TCDC (1.6 mM/15 min)	0.698±0.003	<0.001	100
TUDC (0.2 mM/l20 min)	0.725±0.003	NS	No change
TUDC (1.6 mM/15 min)	0.724±0.001	NS	No change

NS, non significant.

[a] Cells were exposed to two different concentrations of the modulating agents for the time periods shown and then the membrane order parameter '*s*' was determined using electron paramagnetic resonance (EPR) spectroscopy ($n=6$, mean±standard deviation (S.D.). The right-hand column shows, as a percentage, the extent to which the modulators abolish the higher membrane order (increased membrane rigidity) of CH^RC5 cells compared with AUXB1 ($s=0.698\pm0.003$).

[b] Versus control.

effects on cell morphology which could be seen under the light microscope.

4. Discussion

The objective of this study was to examine, with the aid of two bile salt probes, TCDC and TUDC, and an EPR-reporter molecule, 5-doxyl-stearic acid, if structural perturbations in a specific region of the plasma membrane and alteration in membrane order are correlated with P-gp function. As cell material we had available the CH^RC5 and AUXB1 Chinese hamster cells in which we could demonstrate a markedly higher plasma membrane rigidity in the resistant CH^RC5 cells compared with the sensitive AUXB1 cells. Being stereoisomers, TCDC and TUDC differ structurally only in the spatial location of the hydroxyl group at C-7. At the concentrations employed here, TUDC stabilises membranes and TCDC produces perturbations without impairment of bilayer morphology. We have thus been able to observe the effect of localised changes in structure in the apolar domain of the lipid bilayer on P-gp function. We could demonstrate that TCDC lowers membrane order in CH^RC5 cells and abolishes the difference in membrane rigidity between CH^RC5 cells and the parental AUXB1 cell line. TUDC, on the other hand was without effect on the membrane rigidity of CH^RC5 cells. Since we have confirmed that our CH^RC5 cells contain large amounts of P-gp, the ability of the TCDC probe to sensitise these cells to the P-gp substrates and not cisplatin appears to be due to an increased fluidity in a critical domain of the bilayer causing concomitant impairment of P-gp function.

The mechanism by which these probes interfere with membrane structure in Chinese hamster ovary cells may be similar to that reported for hepatocytes. In experiments on liver cell membranes, the strongly hydrophobic TCDC inserts through the polar interface into the inner apolar domain of the bilayer, causes mobilisation

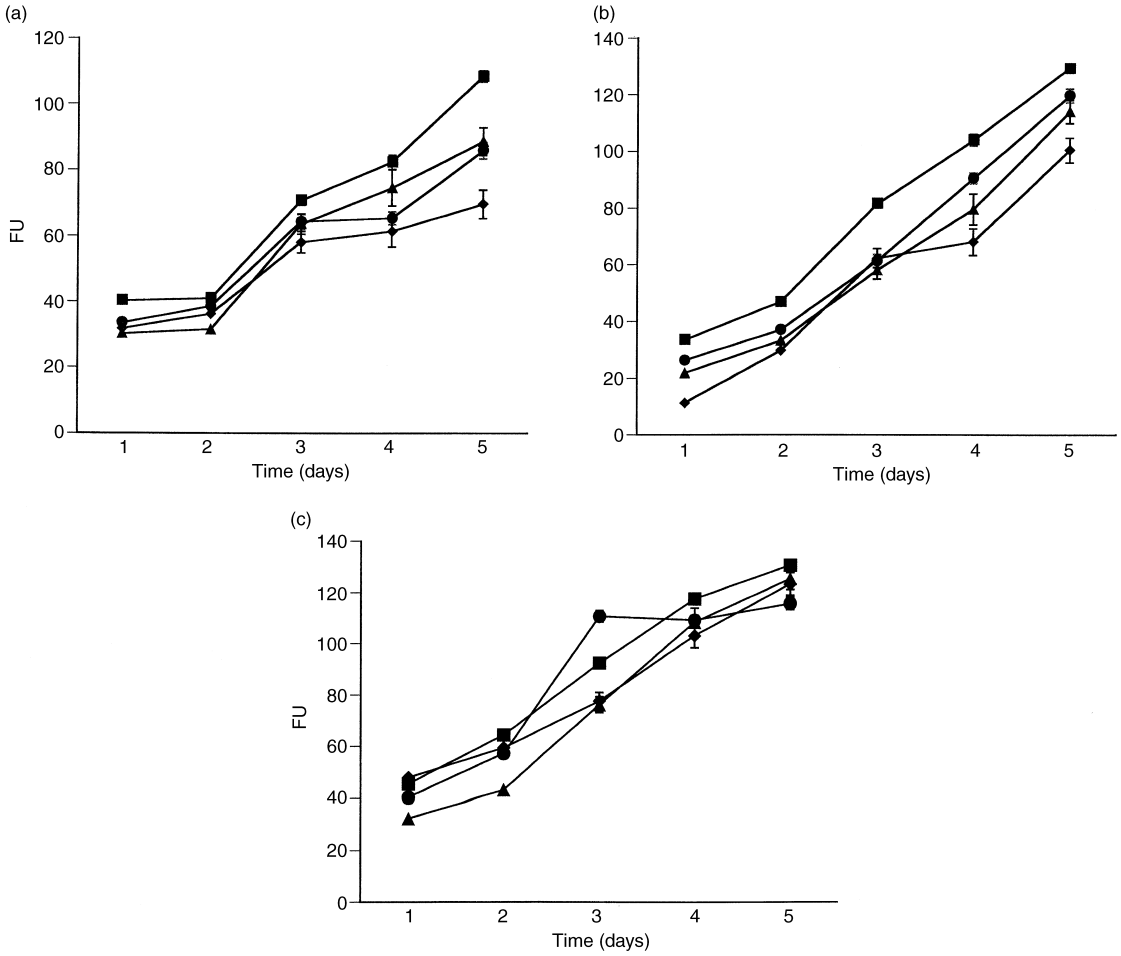

Fig. 3. Cell proliferation curves over 5 days for the CH^RC5 cell-line in the presence of cisplatin (0.5 μg/ml) expressed as fluorescence units (FU): (a) effect of R-verapamil (3 μM): (■) control, (●) cisplatin, (▲) 3 μM R-verapamil, (◆) combination of cisplatin and 3 μM R-verapamil; (b) effect of TCDC (1 mM): (■) control, (●) cisplatin, (▲) 1 mM TCDC, (◆) combination of cisplatin and 1 mM TCDC; (c) effect of TUDC (3 mM): (■) control, (●) cisplatin, (▲) TUDC, (◆) combination of cisplatin and 3 mM TUDC.

of cholesterol and phospholipid and thereby increases fluidity [17]. Studies with the hydrophilic TUDC show accumulation of the steroid nucleus in the apolar region of the bilayer and the taurine fixed near the polar surface aligning the molecule in the interface domain near the phospholipid head-groups. This results in membrane stabilisation in this region, but without concomitant increases in fluidity [17–19].

Earlier studies with TCDC and mitochondrial membranes [28–30] showed that an increased membrane rigidity results in increased permeability or leakiness. It was suggested in these reports that permanent holes are present in rigid plasma membranes permitting passive movement of drug molecules, both in and out of the cell, and therefore causing a reduction or even breakdown of drug-concentration gradients. Modulation of P-gp substrates could therefore involve a mechanism like this in addition to a direct binding of the chemosensitiser to the P-gp molecule. If this is true it could account for the absence of structure-activity relationships for resistance-modifiers.

Lipid depletion studies using bilayer vesicles from CH^RC5 cells have also pointed to the importance of the structural lipid components for P-gp function [31]. P-gp is an integral component of the plasma membrane bilayer with a glycoprotein chain directed outside the cell and ATP-binding regions near the inner surface of the bilayer. When isolated from the plasma membrane and delipidated, P-gp is completely devoid of ATPase activity, but this can be restored by lipid mixtures or by reconstituting into lipid bilayers, thus showing conclusively that the activity of P-gp is absolutely dependent on the integrity of a lipid-rich domain. P-gp is not the only transporter to be affected by a physical phenomenon like this. The ATP-dependent transporter, Na^+-K^+-ATPase, in hepatocyte plasma membranes is also markedly inhibited by TCDC at concentrations similar to those used here [32,33].

The investigations here have demonstrated that the classical resistance modifier R-verapamil, like the bile salt TCDC, also increases plasma membrane fluidity and this action may be in addition to, or related to its ability to cause conformational changes in the P-gp molecule [2,34]. In the recently reported study with the human bladder carcinoma cell lines, RT 112-P and RT 112-$DXR_{8,}$ both R-verapamil and the fatty acid, linolenic acid, caused reversal of resistance and an increase in the fluidity of the cell membrane [14]. Indeed, R-verapamil is lipophilic, accumulates in the inner apolar membrane and the steric effects of the isopropyl side chains may weaken van-der-Waals forces between lipids resulting in a more fluid lipid bilayer. Ionic forces may also be involved since R-verapamil, like a number of other chemosensitisers, is basic in character and can interact with the 'negatively-charged' membrane lipids in areas of the bilayer where it accumulates [6].

In conclusion, increases in plasma membrane fluidity are correlated with sensitisation effects in multidrug resistance. The structural requirements for such activity are not well understood, but hydrophobic interactions within the lipid bilayer could cause conformational changes of specific membrane bound proteins including P-gp. These findings may be useful in the development of resistance modifiers and in the interpretation of functional tests of drug action in the management of tumour patients.

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References

1. Endicott JA, Ling V. The biochemistry of P-glycoprotein-mediated multidrug resistance. *Ann Rev Biochem* 1989, **58**, 137–171.
2. Yusa K, Tsuruo T. Reversal mechanism of multidrug resisance by verapamil: direct binding of verapamil to P-glycoprotein on specific sites and transport of verapamil outward across the plasma membrane of K562/ADM cells. *Cancer Res* 1989, **49**, 5002–5006.
3. Wadkins RM, Houghton PJ. The role of drug-lipid interactions in the biological activity of modulators of multidrug resistance. *Biochim Biophys Acta* 1993, **1153**, 225–236.
4. Frezard F, Garnier-Suillerot A. Permeability of lipid bilayer to anthracycline derivatives. Role of the bilayer composition and of the temperature. *Biochim Biophys Acta* 1998, **1389**, 13–22.
5. Bornmann WG, Roepe PD. Analysis of drug transport kinetics in multidrug-resistant cells using a novel coumarin-vinblastin compound. *Biochemistry* 1994, **33**, 12665–12675.
6. Drori S, Eytan GD, Assraf YG. Potentiation of anticancer-drug cytotoxicity by multidrugresistance chemosensitizers involves alterations in membrane fluidity leading to increased membrane permeability. *Eur J Biochem* 1995, **228**, 1020–1102.
7. Ramu A, Glaubiger D, Magrath IT, Joshi A. Plasma membrane lipid structural order in doxorubicin-sensitive and-resistant P388 cells. *Cancer Res* 1983, **43**, 5533–5537.
8. Dolderer JH, Zimmer G, Woodcock BG, Bockhorn H, Bickeböller R, Schuldes H. Resistance modulation in CHO cells by R-verapamil and bile salts is associated with physical and chemical changes in the cell membrane. *Int J Clin Pharm Ther* 2000, **38**, 196–203.
9. Callaghan R, van Gorkom LCM, Epand RM. A comparison of membrane properties and compositon between cell lines selected and transfected for multidrug resistance. *Br J Cancer* 1992, **66**, 781–786.
10. Callaghan R, Stafford A, Epand RM. Increased accumulation of drugs in a multidrug resistant cell line by alteration of membrane biophysical properties. *Biochim Biophys Acta* 1993, **1175**, 277–282.

11. Schuldes HJ, Dolderer J, Knobloch J, *et al*. Relationship between plasma membrane fluidity and R-verapamil action in CHO cells. *J Clin Pharmacol Ther* 1998, **36**, 71–73.
12. Sinicrope FA, Dudeja PK, Bissonnette BM, Safa AR, Brasotius TA. Modulation of P-glycoprotein mediated drug transport by alteration in lipid fluidity of rat liver canalicular membrane vesicles. *J Biol Chem* 1992, **267**, 24995–25002.
13. Roepe PD, Wie LY, Cruz H, Carlson D. Lower electrical membrane potential and pH_i homeostasis in multidrug-resistant (MDR) cells: further characterization of a series of MDR cell lines expressing different levels of p-glycoprotein. *Biochemistry* 1993, **32**, 11042–11056.
14. Schuldes H, Dolderer JH, Schoch C, Bickböller R, Woodcock BG. Cytostatic sensitivity and MDR in bladder carcinoma cells: implications for tumor therapy. *J Clin Pharmacol Ther* 2000, **38**, 204–208.
15. Armstrong MJ, Carey MC. The hydrophobic-hydrophilic balance of bile salts. Inverse correlation between reverse-phase high performance liquid chromatographic mobilities and micellar cholesterol-solubilizing capacities. *J Lipid Res* 1982, **23**, 70–80.
16. Schubert R, Schmidt KH. Structural changes in vesicle membranes and mixed micelles of various lipid compositions after binding of different bile salts. *Biochemistry* 1988, **27**, 8787–8794.
17. Güldütuna S, Zimmer G, Imhof M, Bhatti S, You T, Leuschner U. Molecular aspects of membrane stabilization by ursodeoxycholate. *Gastroenterology* 1993, **104**, 1736–1744.
18. Lamcharfi E, Cohen-Solal C, Parquet M, Lutton C, Dupré J, Meyer C. Determination of molecular associations of some hydrophobic and hydrophilic bile acids by infrared and Raman spectroscopy. *Eur Biophys J* 1997, **25**, 285–291.
19. Güldütuna S, Deisinger B, Weiss A, Freisleben HJ, Zimmer G, Sipos P, Leuschner U. Ursodeoxycholate stabilizes phospholipid-rich membranes and mimics the effect of cholesterol: investigations on large unilamellar vesicles. *Biochim Biophys Acta* 1997, **1225**, 265–274.
20. Xiang T, Xu Y, Anderson BD. The barrier domain for solute permeation varies with lipid bilayer phase structure. *J Membr Biol* 1998, **165**, 77–90.
21. Ling V, Thompson LH. Reduced permeability in CHO cells as a mechanism of resistance to coichicine. *J Cell Physiol* 1974, **83**, 103–116.
22. Larsson R, Nygren P. Pharmacological modification of multidrug resistance (MDR) in vitro detected by a novel fluorometric microculture cytotoxicity assay. Reversal of resistance and selective cytotoxic actions of cyclosporin A and verapamil on MDR leukemia T-cells. *Int J Cancer* 1990, **46**, 67–72.
23. Marsh D. Electron spin resonance. In Grell E, ed. *Membrane Spectroscopy. Molecular Biology, Biochemistry and Biophysics*. New York, Springer, 1981, 51–142.
24. Jost PC, Griffith OH. Instrumental aspects of spin labeling. In Berliner U, ed. *Spin Labeling Theory and Applications*. New York, Academic Press, 1976, 251–271.
25. Keith AD, Sharnoff M, Cohn GE. A summary and evaluation of spin labels used as probes for biological membrane structure. *Biochim Biophys Acta* 1973, **300**, 379–419.
26. Gaffney BJ, Lin DC. Spin-label measurement of membrane-bound enzymes. In Martonosi A, ed. *The Enzymes of Biological Membranes*. New York, John Wiley & Sons, 1973, 71–90.
27. Van Blitterwijk WJ, van Hoeven RP, van de Meer BW. Lipid structural order parameters (reciprocal of fluidity) in biomembranes derived from steady-state fluorescence polarization measurements. *Biochim Biophys Acta* 1981, **644**, 323–332.
28. Zimmer G, Freisleben HJ, Fuchs J. Influence of pH on sulfhydryl groups and fluidity of the miochondrial membrane. *Arch Biochem Biophys* 1990, **282**, 309–317.
29. Zimmer G. Overview of the role of lipoate in the enzyme complexes of energy metabolism and reducing equivalents. In Fuchs J, Packer U, Zimmer G, eds. *Lipoic Acid in Health and Disease*. New York, Marcel Dekker, 1997, 67–86.
30. Zimmer G. Oxidative stress, Loose-coupling. In Zimmer G. ed. *Membrane Structure in Disease and Drug Therapy*. New York, Marcel Dekker, 2001, in press.
31. Sharom FJ. Characterisation and functional reconstitution of the multidrug transporter. *J Bioenerg Biomembr* 1995, **27**, 15–22.
32. Emmelot P, Bos CJ. Studies on plasma membranes. 3. Mg2+-ATPase, (Na+-K+-Mg2+)-ATPase and 5′-nucleotidase activity of plasma membranes. *Biochim Biophys Acta* 1966, **120**, 369–382.
33. Scharschmidt BF, Keeffe EB, Vessey DA, Blankenship NM, Ockner RK. In vitro effect of bile salts on rat liver plasma membrane, lipid fluidity, and ATPase activity. *Hepatology* 1981, **1**, 137–145.
34. Gottesmann MM, Pastan I. The multidrug transporter, a double-edged sword. *J Biol Chem* 1988, **263**, 12163–12166.