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Ursodeoxycholate stabilizes phospholipid-rich membranes and mimics the effect of cholesterol: investigations on large unilamellar vesicles

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Abstract

Ursodeoxycholate is used to treat primary biliary cirrhosis and is incorporated into hepatocyte plasma membranes. Its steroid nucleus binds to the apolar domain of the membrane, in a similar position to cholesterol. Therefore the question arises whether ursodeoxycholate has a similar effect on membrane structure and stability as cholesterol. Using differential scanning calorimetry the thermotropic behavior of egg phosphatidylcholine and dimyristoylphosphatidylcholine were studied after incubation with cholesterol or ursodeoxycholate. Large unilamellar vesicles were prepared with cholesterol contents of 0–50%. Following incubation of these vesicles with different amounts of ursodeoxycholate, vesicle stability in a gravitational field was investigated by measuring the phospholipid and cholesterol release. Vesicle size was studied by laser light scattering after incubation with cheno- and ursodeoxycholate, and the release of entrapped carboxyfluorescein was measured by means of fluorescence spectroscopy. Increasing cholesterol diminished the enthalpy of the phase transition in the membrane. Ursodeoxycholate decreased the enthalpy of the phase transition at even lower concentrations. Lipid release from vesicles in a high gravitational field diminished with increasing cholesterol content of the vesicles. Ursodeoxycholate had a comparable effect, which increased as the cholesterol content of the vesicles was decreased. Chenodeoxycholate damaged vesicles, whereas ursodeoxycholate did not. Cholesterol and ursodeoxycholate (below its critical micellar concentration) decreased the carboxyfluorescein release from vesicles induced by chenodeoxycholate. Thus like cholesterol, ursodeoxycholate is incorporated into phospholipid model membranes and reduces the change in enthalpy of the gel to liquid-crystalline phase transition. Like cholesterol ursodeoxycholate also maintains membrane stability and prevents membrane damage induced by mechanical and chemical stress. © 1997 Elsevier Science B.V.

Keywords: Ursodeoxycholate; Cholesterol; Phospholipid-rich membrane; Unilamellar vesicle

Abbreviations: C, cholate; CDC, chenodeoxycholate; CF, carboxyfluorescein; CMC, critical micellar concentration; DC, deoxycholate; DSC, differential scanning calorimetry; EYL, egg yolk lecithin; EPR, electron paramagnetic resonance spectroscopy; LUV, large unilamellar vesicles; UDC, ursodeoxycholate

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1. Introduction

Bile salts are potent detergents which damage cell membranes time- and dose-dependently. The efficiency with which membrane lipids, such as cholesterol and phospholipids, are solubilized in vitro generally increases with increasing bile salt hydrophobicity [1–3]. Damage to hepatocyte plasma membranes by hydrophobic bile salts has been demonstrated in animal experiments [4,5]. Moreover, structural and functional injury of hepatocyte membranes caused by hydrophobic bile salts may play a role in the pathogenesis of cholestatic liver diseases in man [6]. Bile salts may bind to basolateral hepatocyte membranes, to cell organelle membranes, and especially to the outside layer of the canalicular membrane following their excretion. UDC protects against damage caused by hydrophobic bile salts [7,8], which seems in part to depend on the amount of its membrane-bound fraction [9].

Recently it has been shown by EPR that UDC is incorporated into the apolar domain of hepatocyte plasma membranes in a similar way to cholesterol [9].

The incorporation of cholesterol molecules into phospholipid bilayers has a profound effect on the gel to liquid-crystalline state of the membrane [10] and this effect has been widely studied due to its relevance to the function of biological membranes [11]. Therefore the first aim of the present study was to investigate whether UDC influences the gel to liquid-crystalline state of phospholipid membranes in a similar way to cholesterol. When a compound is inserted into phospholipid membranes, it influences or disturbs the cooperativity of the phospholipid molecules and this is reflected in a modification of the endotherms or exotherms [12,13] during phase transitions, e.g., transitions from the liquid crystal to the gel state. DSC is widely employed as a method for detecting phase transitions. The area under the transition peak reflects the energy released or absorbed, and the width of the curve indicates the number of molecules that undergo transition simultaneously.

The second aim of the study was to investigate the stabilizing properties of UDC, cholesterol and phospholipids on phospholipid-rich LUV. This was studied by measuring the release of CF entrapped in the vesicles and by determining the vesicle size by laser light scattering following incubation with CDC and UDC. In addition the lipid release from the membranes was measured following centrifugal forces. If UDC stabilizes membranes to a similar extent as cholesterol, this could be one of the modes of action by which UDC protects basolateral hepatocyte membranes in animal experiments and in vitro.

2. Material and methods

Bile salts were obtained from Calbiochem (San Diego, CA); their purity was $> 99\%$ as determined by thin-layer chromatography. EYL (phosphatidylcholine purity $> 99\%$), dimyristoylphosphatidylcholine (purity $> 99\%$) determined by thin-layer chromatography, cholesterol (purity $> 99\%$), CF and buffer substances were from Sigma (Deisenhofen, Germany).

2.1. Differential scanning calorimetry (DSC)

DSC measures the heat (energy) absorbed (or released) by a sample as it undergoes an endothermic (or exothermic) phase transition. DSC was performed by means of a Mettler TA 3000/DSC 30 instrument equipped with a liquid nitrogen cooling device (Mettler Instrumente AG, Greifensee, Switzerland). Baseline fluctuations were below ± 1 mW at a heating rate of 10 K min^{-1} with no peaks or shifts greater than 0.1 mW. Scans were run at rates of $dT/dt = 3$ K min^{-1}. Enthalpy changes were determined by means of a microcomputer which was integrated into the TA 3000/DSC 30 system. Scans were recorded with a printer type PSM MK II (Print swiss matrix). The transition temperature (T_c) is given in °C. For further characterisation of the transitions the change in enthalpy (ΔH [KJ/mol]) and the broadening of the transition peak expressed as ΔD (the width [mm] at half maximum height/max height [mm]) were plotted against the molar concentration of the respective addition (cholesterol, UDC).

Samples were prepared from stock solutions of 100 mg EYL/ml chloroform, 40 mg cholesterol/ml chloroform, 2 mg UDC/ml methanol. Appropriate amounts were collected to give molar ratios of cholesterol/EYL: 0/100, 10/90, 20/80 (corresponding to 0, 10, 20% cholesterol), and UDC/EYL:

0/100, 5/95, 10/90, 15/85, 20/80 (corresponding to 0, 5, 10, 15, 20% UDC), two series of experiments were performed. Thereafter the solutions were dried down in DSC pans (aluminium, V = 40 μl) under a stream of nitrogen. After evaporation under vacuum for at least 20 h, lipid mass was gravimetrically determined using an electronic microbalance (Mettler M3). Absolute mass was determined to be 3–4 mg. 20 μl buffer (10 mM sodium phosphate buffer (pH 7.4), 0.9% NaCl, 50% glycerin) was added and the pans were sealed. For complete hydration and equilibration the pans were kept at 40°C for 24 h. Reference pans, containing 20 μl of the above buffer, were handled in the same manner. 50% glycerin was added to the buffer as antifreeze. In order to eliminate influences of the antifreeze reagent on the phase transition the concentration was kept constant. Heating curves were driven from −40°C to +40°C, cooling curves from +40°C to −40°C.

In addition the effect of UDC on the phase transition of dimyristoylphosphatidylcholine was investigated. The lipid was mixed with the bile salt to give molar ratios of UDC/dimyristoylphosphatidylcholine: 0/100; 0.1/99.9; 0.5/99.5; 1/99; 3/97; 5/95. The procedure described above for DSC measurements was performed, but without adding of antifreeze. Heating curves were driven from 2°C to 45°C and cooling curves from 45° to 2°C.

2.2. Bile salt solutions

The sodium salts of bile acids were dissolved in sodium phosphate buffer (1 mM, pH 7.4). UDC and CDC were prepared in concentrations of 0.05–10 mM; if necessary the pH was adjusted to 7.4.

2.3. Vesicle preparation

EYL and cholesterol were dissolved in chloroform, the lipid concentration was 20 mg/ml solvent. Molar ratios with a cholesterol content of 0–50% were prepared [14]. 1 ml of the lipid solution was freeze-dried and the lipid film was dissolved in 0.1 mM sodium phosphate buffer (pH 7.4, 25°C). After freeze-thawing 10 times the suspension was passed 20 times through a polycarbonate filter of a defined pore size (100 nm) using an extruder (LiposoFast^{tm}, Avestin Inc., Ottawa, Canada). Vesicle size was determined by laser light scattering (Coulter Nanosizer^{R,} details see below). Vesicles were freshly prepared for each experiment.

2.4. Determination of vesicle stability

Vesicle stability against centrifugal forces was determined using ultracentrifugation. 100 μl of vesicles containing different amounts of cholesterol were incubated with 3 ml of different bile salt solutions (UDC and CDC, 0.1–10 mM in sodium phosphate buffer, pH 7.4) at 25°C. The suspension was shaken gently every 10 min. After 30 min the sample was centrifuged at 100 000 × g for 30 min. Phospholipid and cholesterol concentrations were determined photometrically (Boehringer-Mannheim Test-Kit) in the suspension and in the supernatant. The lipid release (concentration in the supernatant) was calculated as the percentage of the total lipid concentration. LUV in buffer served as the control.

2.5. Influence of bile salts on vesicle size

10 μl of vesicle suspension were incubated with 250 μl UDC or CDC (0.1–10 mM in sodium phosphate buffer, pH 7.4, 25°C) for 10 min. The size of the vesicles was determined by laser light scattering (Coulter Nanosizer^{R}). The lower detection limit was 20 nm and the upper limit was 1000 nm, detection time was 1 min.

2.6. Determination of carboxyfluorescein-efflux

EYL (20 mg/ml) were dispersed in sodium phosphate buffer (10 mM, pH 7.4); 6-CF was added to a final concentration of 100 mM in the buffer. Since this lipid-CF solution would not pass through the polycarbonate filter due to the high viscosity, the liposomes were prepared by probe sonication (Branson Sonifier). Suspensions were sonified 6 times on ice for 5 min under a continuous stream of nitrogen. The CF in the extravesicular space was removed by gel chromatography (Sephadex-G 50). After centrifugation of the vesicle loaded columns at 10 000 × g for 10 min, the eluate was collected [15]. The procedure was repeated 4 times, until the CF was removed. 2 ml bile salt solution (CDC or UDC) were mixed with 5 μl of the vesicle solution. The size of the

sonicated vesicles were checked by laser light scattering and found to be 90 ± 20 nm.

The fluorescence intensity I was monitored at 516 nm (excitation at 492 nm) on a Hitachi 650-10S fluorescence spectrometer. The self quenching effect of CF could be excluded, since the concentrations used for detection were far below the quenching concentrations [15]. The intensity of the total amount of CF I (= 100% value) was determined after complete destruction of the liposomes with 100 μl of a Triton X-100 solution (10% v/v). The percentage of CF released was calculated as follows [15]: %CF released = ((It-Io)/(I -Io)) × 100, where t and o refer to the time of sampling and to the instant when bile salt solution was added to the liposomal suspension. The values of CF efflux after 60, 120 and 240 sec were calculated as % of the total CF fluorescence intensity.

2.7. Reproducibility of data

All data shown are mean ± standard error of at least 3 individual measurements.

Table 1
Physicochemical data of heating scans on the thermotropic phase transition of EYL and dimyristoylphosphatidylcholine (DMPC) with increasing amounts of cholesterol or UDC

Additions [mol%]	Δ H [kJ/mol]	T_c [°C]	ΔD [a]
EYL			
100%	13.0 ± 0.2	−9.2 ± 0.7	0,8 ± 0.05
Cholesterol			
10%	10.1 ± 0.02	−9.6 ± 0.05	1.25 ± 0.07
20%	6.1 ± 0.03	−10.9 ± 0.03	1.59 ± 0.11
UDC			
5%	9.31 ± 0.04	−8.1 ± 0.02	1.10 ± 0.07
10%	8.15 ± 0.02	−8.9 ± 0.04	1.15 ± 0.09
15%	7.08 ± 0.05	−9.8 ± 0.03	1.20 ± 0.10
20%	6.46 ± 0.07	−9.4 ± 0.05	1.48 ± 0.13
DMPC			
100%	23.41 ± 0.13	23.3 ± 0.04	0.07 ± 0.01
Cholesterol			
10%	9.93 ± 0.07	21.7 ± 0.03	0.77 ± 0.02
UDC			
0.1%	22.30 ± 0.15	23.5 ± 0.07	0.07 ± 0.01
0.5%	22.24 ± 0.18	23.5 ± 0.08	0.08 ± 0.02
1%	22.71 ± 0.19	23.3 ± 0.04	0.10 ± 0.02
3%	24.92 ± 0.25	22.5 ± 0.04	0.42 ± 0.03
5%	18.60 ± 0.11	20.3 ± 0.05	3.33 ± 0.14

[a] Values were estimated as described in the text.

3. Results

3.1. DSC measurements

As has previously been described, increasing the cholesterol content diminished the enthalpy of the phase transition of the membrane [10]. The enthalpy (ΔH) of the transition of EYL without cholesterol was about 13 kJ/mol lipid (Table 1). The transition temperature (T_c) shifted only marginally from −9.2°C for EYL without cholesterol, to −10.9°C with 20% cholesterol (Table 1). The width of the transition peak became broader with increasing amounts of cholesterol. 20% cholesterol resulted in a maximum broadening of the peak (ΔD = 1.59 heating scans, ΔD = 1.68 cooling scans) with a 53% reduction of enthalpy in heating scans and 55% reduction in cooling scans. The data obtained from the heating and the cooling scans were similar, although the transition temperature was lower for the cooling curve.

Addition of UDC to EYL resulted in a very similar behavior of the phase transitions. The enthalpy of the transition was decreased by the addition of UDC, even at concentrations of 5–20 mol%. Increasing the amount of UDC to 20% led to a further decrease in ΔH; in the cooling scans by about 55%, and in the heating scans by about 50%. The change in T_c was not significant and was in the range of −9 to −10°C for the heating scans and −12 to −13°C for cooling scans. Increased peak broadening (ΔD) was marked at concentrations of 20% UDC (Table 1).

Addition of UDC to dimyristoylphosphatidylcholine reduced the enthalpy of the transition even at concentrations of 0.1 mol%. The effect on peak broadening (ΔD) could be observed at concentrations of 3%. T_c was 22.5°C, addition of 5% UDC led only to a minimal change in transition temperature to 20.3°C (Table 1, Fig. 1).

3.2. Stability of vesicles

The vesicles used were LUV, data obtained by laser light scattering have shown a homogeneous suspension with a size of 100 ± 10 nm (polydispersity index 3) prior to centrifugation. After centrifugation the supernatant was investigated by laser light

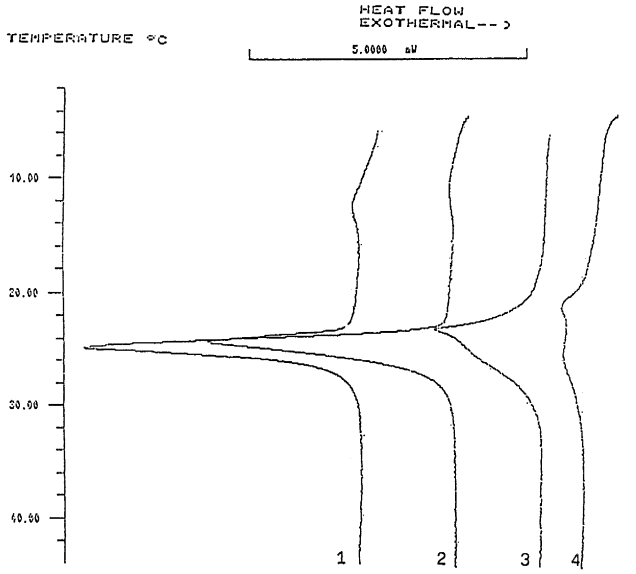

Fig. 1. Heating scans of the phase transition of dimyristoylphosphatidylcholine (DMPC) with increasing amounts of UDC. (1) 100% DMPC; (2) 99% DMPC + 1% UDC; (3) 97% DMPC + 3% UDC; (4) 95% DMPC + 5% UDC.

scattering, particles less < 20 nm in size could be detected. These particles were only induced by centrifugal forces. The stabilizing effect of cholesterol in LUV against centrifugal forces to the vesicles is seen

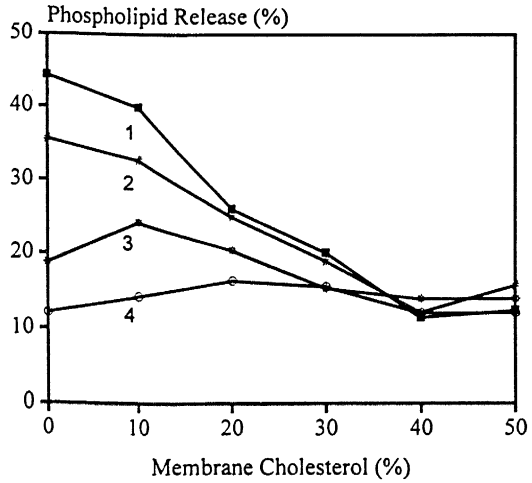

Fig. 2. Effect of UDC on phospholipid release (induced by centrifugal forces: $100\,000 \times g$)) from LUV containing different amounts of cholesterol. (1) Control; (2) incubation with 0.1 mM UDC; (3) incubation with 1 mM UDC; (4) incubation with 3 mM UDC. Mean standard error of triplicate measurements: curve (1) = ±3.7; curve (2) = ±3.4; curve (3) = ±4.1; curve (4) = ±4.0.

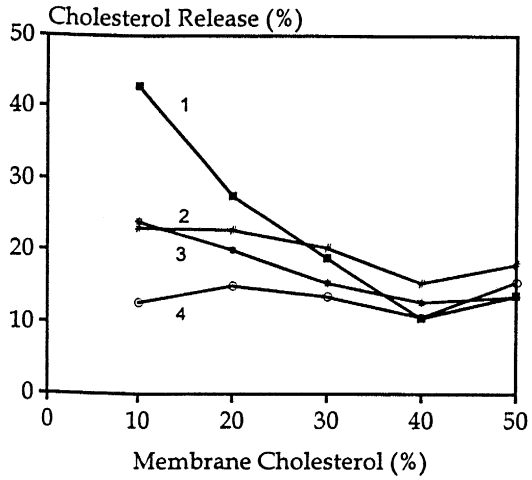

Fig. 3. Effect of UDC on cholesterol release (induced by centrifugal forces: $100\,000 \times g$) from LUV containing different amounts of cholesterol. (1) Control; (2) incubation with 0.1 mM UDC; (3) incubation with 1 mM UDC; (4) incubation with 3 mM UDC. Mean standard error of triplicate measurements: curve (1) = ±4.1; curve (2) = ±5.4; curve (3) = ±2.1; curve (4) = ±3.7.

in Fig. 2 (curve 1). Increasing the cholesterol content of the membrane reduces the phospholipid release. The greatest stabilizing effect was found with membranes containing 40% cholesterol, which reduced the phospholipid release from 44.3 to 11.5%. A further increase in membrane cholesterol did not further enhance the stability of the vesicles since the phospholipid release then increased to 12.5%. The cholesterol release induced by centrifugal forces was also decreased by increasing the membrane cholesterol (Fig. 3, curve 1). The most marked effect was found with 40% membrane cholesterol (cholesterol release 10.6%); in membranes with 10% cholesterol the release was 42.7%.

Similar results were found when the membrane lipid release from LUV was measured after incubation with UDC. The greatest effect of UDC on membrane stabilization was found when 100% EYL vesicles were incubated with 3 mM UDC: a reduction in the phospholipid release from 44.3 to 12.2% was observed (Fig. 2).

Vesicle pelleting by bile salts could be excluded since in control experiments with 3 mM CDC the phospholipid release in the supernatant increased from 44.3 to 91.6%. In addition UDC was used in concentrations below the CMC excluding formation of lipid aggregates.

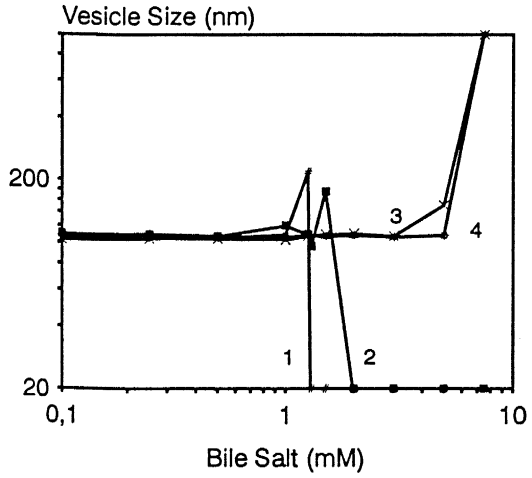

Fig. 4. Influence of UDC and CDC on the size of LUV. (1) Effect of CDC on LUV with 100% EYL and (2) containing 40% of cholesterol. (3) Effect of UDC on LUV with 100% EYL and (4) containing 40% of cholesterol. Mean standard error of triplicate measurements: curve (1) = ±14; curve (2) = ±12; curve (3) = ±21; curve (4) = ±19.

UDC also prevented cholesterol release (Fig. 3). In vesicles containing 10% cholesterol, the cholesterol release was reduced from 42.7% to 23.1% by incubation with 0.1 mM UDC. UDC concentrations up to 3 mM reduced cholesterol release to 12.8%. Vesicles with a cholesterol content over 30% were not further stabilized by UDC (Fig. 3). Increasing the UDC concentration (Fig. 7) above the CMC increased the phospholipid release to 33.8% in 100% EYL vesicles (control value 44.3%), indicating that UDC has no effect on phospholipid and cholesterol release above the CMC of about 5.5 mM [15].

3.3. Determination of vesicle size

The mean size of control vesicles measured by laser light scattering was 100 ± 10 nm (polydispersity index 3). An increase in vesicle size of 50–100% was detected after incubation with CDC in a concentration just below that required for vesicle solubilization (Fig. 4), indicating that the bile salt/lipid ratio in the membrane is increased. 100% EYL vesicles were solubilized by concentrations of 1.1–1.25 mM CDC, i.e., vesicle size was reduced below 20 nm. When the cholesterol concentration of the vesicles was gradually increased, the CDC concentration required for membrane solubilization also increased. In vesicles containing 40% cholesterol, membrane solubilization was found at CDC concentrations of 1.4–1.5 mM. Cholesterol concentrations above 40% did not further increase membrane stability.

In incubation experiments with UDC the size of 100% EYL vesicles remained constant up to concen-

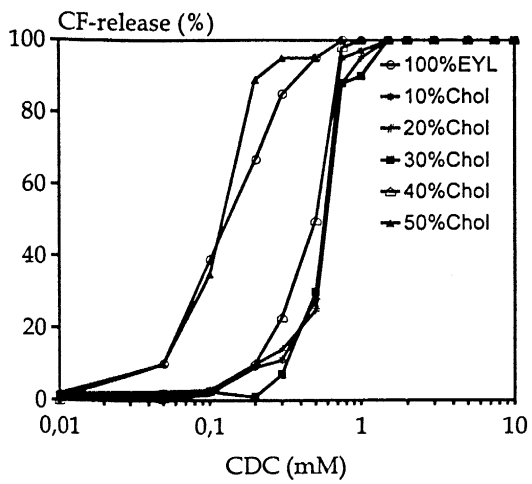

Fig. 5. CF release from LUV with EYL and different cholesterol concentrations induced by CDC. Incubation time with bile salts: 60 sec. Mean standard error of triplicate measurements: curve for 100% EYL = ±7.1.; curve for 10% Chol = ±5.3; curve for 20% Chol = ±4.0; curve for 30% Chol = ±3.7; curve for 40% Chol = ±4.8; curve for 50% Chol = ±6.8.

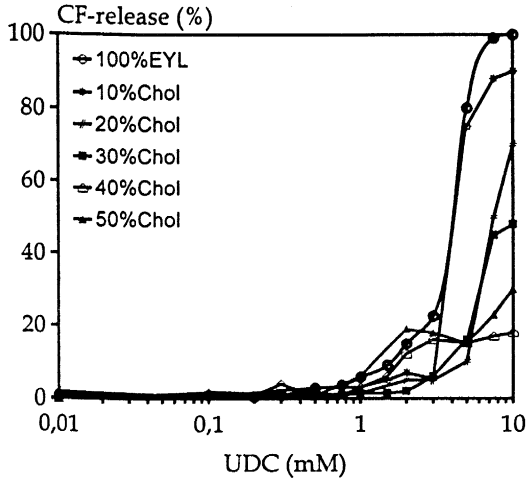

Fig. 6. CF release from LUV with EYL and different cholesterol concentrations induced by UDC. Incubation time with bile salts: 60 sec. Mean standard error of triplicate measurements: curve for 100% EYL = ±6.5.; curve for 10% Chol = ±4.0; curve for 20% Chol = ±4.5; curve for 30% Chol = ±3.1; curve for 40% Chol = ±5.9; curve for 50% Chol = ±7.7.

trations of 5 mM UDC. Above this concentration vesicle size increased dramatically and vesicle aggregation (possibly with the formation of multilamellar vesicles) could be detected with a size greater than 1000 nm (Fig. 4), the polydispersity index was 5. By increasing the cholesterol concentration vesicle aggregation was shifted to higher UDC concentrations (7.5 mM UDC).

3.4. CF efflux

In preliminary experiments we investigated the effect of bile salts on CF efflux for incubation times up to 60 min. Equilibration of the system was reached after 4–5 min, longer incubation times revealed no more information on membrane permeability. The most dramatic changes in membrane permeability could be observed in the first minutes during bile salts incubation. The percentage of CF in the supernatant after 60, 120 and 240 s incubation with CDC and UDC revealed a time-dependent increase of CF efflux from EYL vesicles. In 100% EYL vesicles CDC induced 10% CF efflux even at concentrations below 0.05 mM. The CF efflux depended on the cholesterol concentration of the vesicles (Fig. 5). Increasing the cholesterol concentration decreased the CF efflux. This effect was found up to cholesterol concentrations of 40%. At cholesterol concentrations above 40%, the CF efflux caused by bile salts increased again.

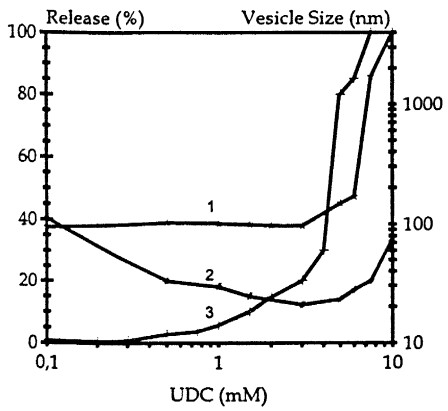

Fig. 7. Influence of UDC on 100% EYL-LUV: (1) On vesicle size, (2) on phospholipid release and (3) on CF release. Mean standard error of triplicate measurements: curve (1) = ±21; curve (2) = ±3.9; curve (3) = ±6.5.

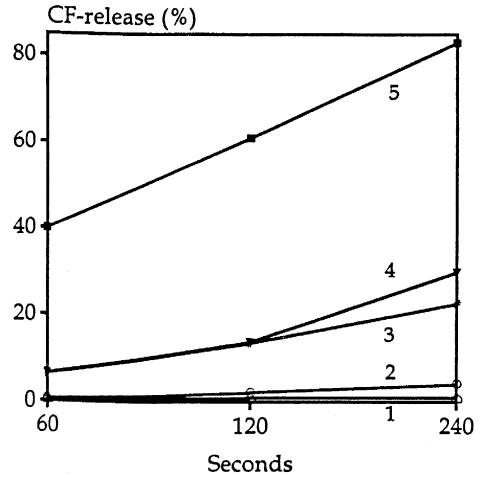

Fig. 8. CF release from LUV with 100% EYL. (1) Control; (2) Incubation with 0.1 mM UDC; (3) Incubation with 0.25 mM UDC and 0.1 mM CDC; (4) Incubation with 0.1 mM UDC and 0.1 mM CDC; (5) Incubation with 0.1 mM CDC. Mean standard error of triplicate measurements: curve (1) = ±5.9; curve (2) = ±6.2; curve (3) = ±8.7; curve (4) = ±7.5; curve (5) = ±8.3.

Below 4 mM UDC induced only minor CF release occurred from 100% EYL vesicles (Fig. 6). Incubation with 2 or 3 mM UDC induced a CF release of 9–18%. In concentrations below the CMC of UDC, vesicle size remained constant and membrane lipids were not released (Fig. 7). However, a distinct release of CF (80% and more) was observed with concentrations above 4 mM UDC (Figs. 6 and 7).

The release of CF from 100% EYL vesicles induced by 0.1 mM CDC was $>80\%$ inhibited by incubation with 0.1 mM UDC (Fig. 8). A similar effect could be achieved by increasing the membrane cholesterol (Fig. 5). The inhibitory effect of UDC was not seen in vesicles containing cholesterol (data not shown).

4. Discussion

By means of electron paramagnetic resonance spectroscopy and with radioactively labelled bile salts, it has been shown that UDC is incorporated into hepatocyte plasma membranes [9] and LUV [16]. The steroid nucleus of UDC is located in the apolar part of the lipid bilayer decreasing membrane polarity and preventing membrane damage caused by hydrophobic

bile salts. Since in isolated cell membranes UDC molecules occupy a similar position to cholesterol [9], the aim of the present study was to compare the effects of UDC and cholesterol on membrane stabilization using large unilamellar vesicles as a membrane model.

Cholesterol is known to reduce the enthalpy of the phase transition of phospholipid membranes [10,13]. DSC revealed an effect of UDC on the phase transition that was comparable to that of cholesterol. Both compounds progressively reduced the enthalpy of the transition as their concentrations were increased. Enthalpy was reduced by about 50% when the UDC:phospholipid or cholesterol:phospholipid ratio in the model membranes was as low as 1:5. In these experiments glycerol was added as antifreeze, because of the low transition temperature of the lipid. In experiments with dimyristoylphosphatidylcholine, however, the effect of antifreeze on the phase transition was excluded and again an inhibitory effect UDC on the enthalpy of the phase transition could be observed, even at concentrations of 0.1%.

In DSC studies with taurocholate [17] and taurodeoxycholate [18] the transition temperature of DPPC was changed, indicating that micellar structures affecting the cooperativity of the lipid. On the contrary UDC did not change under our experimental conditions the transition temperature of the lipid. Cholesterol interacts with membranes and affects their thermotropic properties by inserting into the hydrophobic part of the membrane. This leads to a broadening, a reduction, or an elimination of the phase transition without altering the phase transition temperature as has been previously reported in the literature [13,19]. The thermal behaviour of a lipid is an important factor in the permeability, fusion and protein binding of lipid membranes [11,20]. The similar effects of UDC and cholesterol on the phase transition therefore indicate that these substances may interact with lipids in a similar fashion. UDC interacts with membrane lipids, ordering the chains and decreasing their motion as does cholesterol [21]. We were unable to obtain data on order parameters using EPR, since it was not possible to remove the bile salts from the vesicle containing suspension without destroying the membranes and previous studies have shown that bile salts interact with lipid spin labels. However, if bile salts are removed from the supernatant, UDC decreases membrane polarity and fluidity [9]. Thus the DSC experiments complement our EPR investigations.

Interactions between bile salts and phospholipid membranes have also been described for C, DC and CDC [22,23]. By means of NMR experiments [22] it has been shown that C molecules are most probably placed flat on the membrane surface rather than intercalated between the phospholipid molecules. However, deuterated DC and CDC, were found to be incorporated perpendicularly into the surface of the lecithin bilayer membrane [23]. The effect of bile acids on membranes is induced by membrane binding [16]. The protonated form of the bile acids in the membranes depends on the pKa [16,24], more polar bile acids, e.g., taurine conjugated are expected not to be protonated in the membrane. The pKa of UDC is higher than CDC [25] and in membranes is increased by several pH units [24]. Under the experimental conditions used in this study UDC is expected to be protonated and membrane-bound. The steroid nucleus of UDC is located in the center of the bilayer, the polar carboxyl group oriented towards the aqueous phase, while glyco- and tauroursodeoxycholate are located in the interface, oriented with their polar side chains on the membrane surface [9]. Since UDC is a relatively hydrophilic bile salt as estimated with reversed phase high performance liquid chromatography, incorporation could occur as a paired molecule, thereby avoiding contact between its polar groups and the fatty acid chains of the membrane [26].

Cholesterol is incorporated into the membrane with its hydroxyl group oriented to the aqueous surface and the aliphatic chain aligned parallel to the acyl chains in the center of the bilayer [11,20]. The steroid nucleus is positioned along the first ten carbons of the phospholipid chain. EPR studies suggest that the steroid nucleus of UDC is located a few atoms deeper than that of cholesterol, but in principle in the same domain [9].

Although the steroid nucleus of UDC and cholesterol are localized in the same domain of the plasma membrane and decrease membrane fluidity, incubation of CF loaded vesicles with UDC below the CMC induces slight CF release of about 4–18%. However, by measuring the vesicle size, the polydispersity index and the phospholipid release from the membranes, a disruption of the vesicles and the formation

of mixed phospholipid/bile salt micelles was excluded (Fig. 6). It has been suggested that during incubation of vesicles with bile salts, the number of molecules in the outer leaflet of the bilayer increases, and that the difference in the number of molecules in the outer and inner leaflet results in a sudden membrane foldover accompanied by a transient pore formation [16]. After redistribution of UDC in both leaflets of the bilayer, membranes were rapidly stabilized and the CF release ceased. This could be an explanation for the CF release in our experiments. In the case of CDC, membrane foldover and pore formation may also be caused by interactions between the hydroxygroups of the steroid nucleus.

CDC above the CMC solubilizes phospholipid membranes to form mixed micelles with membrane lipids [27], whereas low concentrations of UDC stabilize LUV membranes as does cholesterol. The stabilizing effect of UDC was inversely correlated to the cholesterol content of the membrane. No effect of UDC was observed in vesicles with a membrane cholesterol content of 30% and more. This phenomenon may be due to the condensation of the membrane by cholesterol molecules reducing the fluidity of the phophoslipid layers above the phase transition temperature [11,20], thus preventing the penetration of bile salt molecules [16].

Previous investigators [28] have described a protective effect of tauroursodeoxycholate in concentrations of up to 15 mM against the damaging effect of taurodeoxycholate on vesicles containing 50% cholesterol. Above the CMC [29,30] we obtained similar data (not shown) for UDC. Above the CMC UDC induced fusion of vesicles to multilamellar aggregates (Fig. 7). Lipid aggregation was also shown for taurocholate above the CMC [31]. This phenomenon may be induced by the formation of apolar clusters on the membrane surface. Above a cholesterol/lipid ratio of 1, cholesterol also induced vesicle fusion [21].

In low concentrations CDC increased the permeability of the vesicles as shown by the CF release. UDC also had a protective effect against CDC damage, when vesicles were incubated in an equimolar UDC/CDC-solution (0.1/0.1 mM). This effect could be due to a molecular interaction of bile salts in the buffer milieu, as shown previously [9], or UDC could impede the penetration of CDC into the inner leaflet of the bilayered membrane. The latter may be more likely, since if the effect were merely a molecular interaction in the incubation medium, the inhibition of the CF release should be independent of the lipid composition of the vesicles. However, since there was no relevant inhibition of CF release by UDC in cholesterol containing vesicles, a direct membrane effect can be assumed. As shown above, this direct membrane effect of UDC is inversely correlated to the cholesterol concentration of the vesicles inhibiting the insertion of UDC [16]. Thus membrane-bound UDC impedes the penetration of cholesterol into the membrane.

The similarities between UDC and cholesterol concerning their interaction with phospholipid membranes may be of relevance for the therapeutic effect of UDC. In cholestatic liver diseases liver plasma membranes (especially canalicular hepatocyte membranes) may change their lipid composition [32], since during cholestasis the more hydrophobic 'toxic' bile salts seem to accumulate around the bile capillaries [33,34]. Since bile secretion in these patients is reduced and retention of the more apolar, toxic bile salts into blood occurs, similar alterations may occur at basolateral hepatocyte membranes. One of the therapeutic effects of UDC could be the maintenance of membrane structure, stability and function.

References

[1] A.F. Attili, M. Angelico, A. Cantafora, D. Alvaro, L. Capocaccia, Med. Hypoth. 19 (1986) 57–69.
[2] S.G. Barnwell, P.J. Lowe, R. Coleman, Biochem. J. 216 (1983) 107–111.
[3] C.H. O' Connor, R.G. Wallace, K. Iwamoto, T. Taguchi, J. Sunamoto, Biochim Biophys Acta 817 (1985) 95–102.
[4] F. Schaffner, N.B. Javitt, Lab. Invest. 15 (1966) 1783–1792.
[5] K. Miyai, V.M. Price, M.M. Fisher, Lab. Invest. 24 (1971) 292–302.
[6] H. Greim, D. Trülzsch, P. Czygan, J. Rudick, F. Hutterer, F. Schaffner, H. Popper, Gastroenterology 63 (1972) 846–850.
[7] K. Kitani, M. Ohta, S. Kanai, Am. J. Physiol. 248 (1985) G407–417.
[8] D.M. Heuman, W.M. Pandak, P.B. Hylemon, Z.R. Vlahcevic, Hepatology 14 (1991) 920–926.
[9] S. Güldütuna, G. Zimmer, M. Imhof, S. Bhatti, T. You, U. Leuschner, Gastroenterology 104 (1993) 1736–1744.
[10] B.D. Ladbrooke, R.M. Williams, D. Chapman, Biochim. Biophys. Acta 150 (1968) 333–340.

[11] R.A. Demel, B. De Kreuyff, Biochim. Biophys. Acta 457 (1976) 109–132.
[12] J. Suurkuusk, B.R. Lentz, R.L. Barenholz, T.E. Thompson, Biochemistry 15 (1976) 1393–1401.
[13] T.P.W. McMullen, L.N.A.H. Ruthen, R.N. McElhaney, Biochemistry 32 (1993) 516–522.
[14] R.C. Mc Donald, R.I. Mc Donald, B.Ph.M. Menco, K. Takeshita, N.K. Subbarao, L.R. Hu, Biochim. Biophys. Acta 1061 (1991) 297–302.
[15] J.N. Weinstein, E. Ralston, L.D. Leserman, R.D. Klausner, P. Dragsten, P. Henkart, R. Blumenthal, in: G. Gregoriadis (Ed.), Liposome Technology, Vol. III, CRC Press, Florida, 1984, pp. 183–204.
[16] R. Schubert, K.H. Schmidt, Biochemistry 27 (1988) 8787–8794.
[17] C.H. Spink, V. Lieto, E. Mereand, C. Pruden, Biochemistry 30 (1991) 5104–5112.
[18] C.H. Spink, K. Müller, J.M. Sturtevant, Biochemistry 21 (1982) 6598–6605.
[19] J. Hernandez-Borell, K.M.W. Keough, Biochim. Biophys. Acta 1153 (1993) 277–282.
[20] P.L. Yeagle, Biochim. Biophys. Acta 822 (1985) 267–287.
[21] M.C. Phillips, Hepatology 12 (1990) 75S–82S.
[22] J. Ulmius, G. Lindblom, H. Wennerström, L.B. Johansson, K. Fontell, O. Södermann, G. Arvidson, Biochemistry 21 (1982) 1553–1560.
[23] H. Saito, Y. Sugimoto, R. Tabeta, S. Suzuki, G. Izumi, M. Kodama, S. Toyoshima, C. Nagata, J. Biochem. (Tokyo) 94 (1983) 1877–1887.
[24] H. Igimi, M.C. Carey, J. Lipid. Res. 21 (1980) 72–90.
[25] F. Kamp, J.A. Hamilton, Biochemistry 32 (1993) 11074–11086.
[26] M.C. Carey, P.F. Lindley, in: X International Bile Acid Meeting, Poster Abstracts, Freiburg 80, 1988.
[27] D.E. Cohen, M. Angelico, M.C. Carey, J. Lipid. Res. 30 (1990) 5570.
[28] D.M. Heuman, R. Dajaj, Gastroenterology 106 (1994) 1333–1341.
[29] A. Roda, A.F. Hofmann, K.J. Mysels, J. Biol. Chem. 258 (1993) 6362–6370.
[30] M.C. Carey, J.-C. Montet, M.C. Phillips, M.J. Armstrong, N.A. Mazer, Biochemistry 20 (1981) 3638–3647.
[31] T.E. Little, S.P. Lee, H. Madani, E.W. Kaler, K. Chinn, Am. J. Physiol. 260 (1991) G70–G79.
[32] D.J. Smith, E.R. Gordon, J. Hepatol. 5 (1987) 362–365.
[33] T. Okishio, P.P. Nair, Biochemistry 5 (1966) 3662–3668.
[34] S.G. Barnwell, P.J. Lowe, R. Coleman, Biochem. J. 216 (1983) 107–111.