JPP JOURNAL OF Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY
JPP Journal of Pharmacy And Pharmacology
Research Paper

Anomalous interaction of tri-acyl ester derivatives of uridine nucleoside with a L-α-dimyristoylphosphatidylcholine biomembrane model: a differential scanning calorimetry study

Jhon Fernando Berrío Escobar[a], Diana Margarita Márquez Fernández[a], Cristiano Giordani[a,b], Francesco Castelli[c] and Maria Grazia Sarpietro[c]

[a]Productos Naturales Marinos, Departamento de Farmacia, Facultad de Ciencias Farmacéuticas y Alimentarias, [b]Instituto de Física, Universidad de Antioquia, UdeA, Medellín, Colombia and [c]Dipartimento di Scienze del Farmaco, Università degli Studi di Catania, Catania, Italia

Keywords
anticancer nucleoside; biomembrane models; differential scanning calorimetry; fatty acid; uridine

Correspondence
Maria Grazia Sarpietro, Dipartimento di Scienze del Farmaco, Università degli Studi di Catania, Viale A. Doria 6, 95125 Catania, Italy.
E-mail: mg.sarpietro@unict.it

Received June 25, 2018
Accepted October 19, 2018

doi: 10.1111/jphp.13038

Abstract

Objectives Uridine was conjugated with fatty acids to improve the drug lipophilicity and the interaction with phospholipid bilayers.
Methods The esterification reaction using carbodiimides compounds as coupling agents and a nucleophilic catalyst allowed us to synthesize tri-acyl ester derivatives of uridine with fatty acids. Analysis of molecular interactions between these tri-acyl ester derivatives and L-α-dimyristoylphosphatidylcholine (DMPC) multilamellar vesicles (MLV) – as a mammalian cell membrane model – have been performed by differential scanning calorimetry (DSC).
Key findings The DSC thermograms suggest that nucleoside and uridine triacetate softly interact with phospholipidic multilamellar vesicles which are predominantly located between the polar phase, whereas the tri-acyl ester derivatives with fatty acids (myristic and stearic acids) present a strongly interaction with the DMPC bilayer due to the nucleoside and aliphatic chains parts which are oriented towards the polar and lipophilic phases of the phospholipidic bilayer, respectively. However, the effects caused by the tri-myristoyl uridine and tri-stearoyl uridine are different.
Conclusions We show how the structural changes of uridine modulate the calorimetric behaviour of DMPC shedding light on their affinity with the phospholipidic biomembrane model.

Introduction

The anticancer nucleoside and analogues drugs have been widely used for cancer treatment since the 1960s, against solid (e.g. lung, liver and breast) and soft (e.g. leukaemia and non-Hodgkin lymphoma) tumours. They show cytostatic, cytotoxic and antiproliferative activities due to their action mechanisms as antimetabolites for the DNA (alpha and epsilon polymerase inhibition) and RNA synthesis, and for the purine and pyrimidine nucleosides synthesis. Their incorporation into DNA chain causes demethylation and hypomethylation of fragments. Moreover, some nucleoside drugs can show activation of apoptosis routes on cytoplasm and mitochondrial side.[1–6]

Anticancer nucleosides present numerous pharmacokinetic problems, such as low selectivity in attacking cancer cells, and an extracellular inactivation and degradation due to their high chemical and biological lability. They also show very pharmacodynamics disadvantages, some of these are as follows: low cellular uptake, intracellular inactivation and biological degradation by means of ecto-nucleotidase and nucleoside deaminase enzymes.[2,4–7] Due to the great disadvantages revealed, the nucleoside drugs have a very short plasma half-life and very low oral bioavailability; therefore, these drugs type should be administered in higher doses and intravenously to be effective. This administration form is responsible for severe side effects as gastrointestinal disorder, neurotoxicity, nephrotoxicity and

hepatotoxicity, among others, caused for the significant cell death showed on healthy tissues adjacent to tumour or in other organs.[2,4–8] Due to the disadvantages and adverse effects, a substantial number of studies have been focused on improving the biological and physico-chemical properties of nucleoside drugs, through the synthesis of aliphatic derivatives with short and long aliphatic acids chains, aromatic derivatives and conjugates synthesized with hydroxyl substances of big molecular weight as polymer and sterols. These derivatives can provide protection against enzymatic or chemical degradation and enhance the cellular uptake or transport across cell membranes[2,4,7–11] Other investigations have been focused on the search of new nucleosides or analogues that exhibit a better physico-chemical and/or biological characteristic. It has been found that uridine, an essential nucleoside, and some natural derivatives with oligosaccharide or phosphate as substituents, among others, show cytotoxic activities to diverse tumour cell lines to higher concentrations than physiological levels of these substances. Some of these compounds behave as antimetabolites, interrupt the essential nucleoside and RNA synthesis or act as inhibitors of glycosyltransferases enzymes.[12–14] Other contributions based on the use of synthetic derivatives of the same nucleoside, with aliphatic substituent and/or halogen groups, ester with aliphatic acids of long chains and aromatic acids, and conjugates with triterpenoids hydroxylated, have showed significant cell viability inhibition against solid and soft tumours cell lines.[15–17] Therefore, in the oncologic therapy is highly desired to obtain novel drugs or enhanced anticancer drugs that exhibit improvement on pharmacological or biological properties, to be used in chemotherapy.[18]

It is noteworthy to point out that the tri-acyl ester derivative of uridine with acetic acid, used in chemotherapy in combination with antiviral (stavudine) and anticancer (capecitabine) nucleoside drugs, has shown a decreasing of the side effects (in terms of neurotoxicity showed by the antiviral drug and gastrointestinal disorder and cardiotoxicity, presented by the anticancer nucleoside) exhibited by these drugs when employed in high doses or administrated for longer times, whereas the anticancer and antiviral activities remain unaltered. The uridine triacetate is a drug (namely Vistogard®) approved by The US Food and Drugs Administration (FDA) and used in the treatment of overdoses and numerous life-threatening toxicities from the capecitabine anticancer nucleoside.[14,18]

Studies on the interaction between cell membranes and anticancer drugs (or prodrugs), are very interesting for several reasons, including the understanding of the mechanism of action, the miscibility process, the diffusion through the phospholipidic bilayer structures, as well as the membrane structural changes induced by the substances evaluated. However, the analysis of interactions of drugs (or prodrugs) with biological membranes is not straightforward since cellular membranes are chemically and physico-chemically very complex.[19–23] Although very simplified compared to the complexity of biological cell membranes, lipid bilayers and monolayers can be employed as useful models. The biomembrane models permitted to investigate the nature of the interaction between phospholipids and drugs or compounds with biological activity.[24–26] The L-α-dimyristoylphosphatidylcholine (DMPC) is a saturated phospholipid that is found in high quantities as component of healthy and tumour cell membranes of mammalian. It easily builds phospholipid structures as monolayer and liposomes, and the main transitions are under of 37 °C, which is the human corporal temperature; therefore, the DMPC is widely used as phospholipid for the biomembrane models.[19,21,22]

The analysis of the diverse types of the interaction involved between DMPC and drugs can be performed by differential scanning calorimetry (DSC),[7,27] a technique widely employed in several studies, because the thermodynamic properties and technical parameters can be accurately and easily monitored. The procedure for the formation of liposomes is relativity simple and allows to use phospholipids of several types as biomembrane models. Moreover, this method is trustworthy in revealing details of the diverse types of interactions of drugs, prodrugs and biologically active substances of different polarity with diverse phases of MLV; therefore, offering information about the miscibility and distribution of the compounds analysed over the phospholipid structures.[20,24,25]

Here, we prepared DMPC-MLV as a simplified model of biomembrane and we studied, by DSC, the interactions between uridine and the nucleoside derivatives and the phospholipid bilayers.

Materials and Methods

Chemicals

All solvents employed were of reactive grade. The following substances were used for the synthesis: uridine 99% (Alfaesar, Karlsruhe, Germany) as substrate; acetic anhydride, myristic acid and stearic acid 99% (Merck, Darmstadt, Germany) were employed as substituents; *N,N′*-diciclohexilcarbodiimide (DCC) 99% (Alfaesar, Karlsruhe, Germany), and 4-*N,N*-dimethylamino-pyridine (DMAP) 99% (Alfaesar, Karlsruhe, Germany) as coupling agent and nucleophilic catalyst, respectively. L-α-dimyristoylphosphatidylcholine (DMPC) 99% (Genzyme Pharmaceuticals, Liestal, Switzerland) and tris-hydroximethyl-aminomethane (TRIS) (Merck, Darmstadt, Germany) were employed for the preparation of phospholipidic multilayer vesicles (MLV). The reaction mixture was monitored by TLC on F254 silica gel (Merck, Darmstadt, Germany), the

products were purified by chromatography column normal phase, some compounds were re-purified by preparative TLC on normal phase, as reported elsewhere.[16,17]

The tri-acyl ester derivatives of uridine isolated, were characterized by: mass spectrometry, recorded on Agilent 6300-LC/MS Equipment (electrospray ionization (ESI) and Ion-Trap analyzer) and an Agilent 6100 Series-LC/MS (ESI and single quadrupole analyzer), both analyses in positive mode and direct injection method, and nuclear magnetic resonance spectroscopy (NMR) unidimensional and bidimensional spectra, recorded on a Bruker 300 NMR spectrometer, with tetramethylsilane (TMS) employed as internal standard. The $CDCl_3$ (99% deuterium purity, Merck) was used as solvent for the (3′,4′,6′-*O*-trimyristoyl-uridine and 3′,4′,6′-*O*-tri-stearoyl-uridine) and dimethylsulfoxide (DMSO-*d6*, 99% deuterium purity, Merck) employed as solvent for the 3′,4′,6′-*O*-triacetyl-uridine, as reported elsewhere.[16,17]

Synthesis of tri-acyl uridine derivatives

3′,4′,6′-O-triacetyl uridine or uridine triacetate

The uridine and nucleophilic catalyst (DMAP) were dissolved in a solution of acetic anhydride/DMF/TEA, the mixture was stirred at room temperature (the detailed conditions of synthesis are reported elsewhere.[17]). The product was isolated as a yellow solid (quantitative yield), melting point: 127–128 °C. ^{1}H NMR (300 MHz; DMSO-*d6*), δ ppm (multiplicity; integration; *J* (Hz); position): 2.04 (d; 6H; H-2″ acetyl linked to O-3′ and O-6′ ribose ring), 2.07 (s; 3H; position H-2″ acetyl linked to O-4′ ribose ring), 4.25 (m; 3H; H-6′ and H-5′ ribose ring), 5.33 (m; 1H; H-4′ ribose ring), 5.44 (m; 1H; H-3′ ribose ring), 5.72 (d; 1H; *J* = 8.0; H-5 uracil ring), 5.87 (d; 1H; *J* = 5.1; H-2′ ribose ring), 7.69 (d; 1H; *J* = 8.1; H-6 uracil ring). ^{13}C NMR spectroscopy data and 2D correlations as reported previously.[17] ESI-MS (positive mode) m/z: Calculated for $C_{15}H_{18}N_2O_9$: 370.3114; found, 393.1 $[M+Na]^+$.

3′,4′,6′-O-trimyristoyl uridine and 3′,4′,6′-O-tris-tearoyl uridine

The synthesis of the tri-acyl ester derivative of uridine with fatty acids (myristic acid and stearic acid) was performed as reported elsewhere.[16,17] The fatty acid and coupling agent (DCC) were dissolved in a DMF/chloroform mix, after the nucleoside (300 mg) and DMAP (catalyst amount) were added to reaction mixture. Detailed information of synthesis as reported elsewhere.[17]

3′,4′,6′-*O*-trimyristoyl uridine was isolated as a white solid (20% yield), melting point: 63–64 °C. ^{1}H NMR ($CDCl_3$), δ ppm (multiplicity; integration; *J* (Hz); position): 0.92 (t; 9H; *J* = 6.6; -CH3; H-14″ myristoyl chain), 1.32 (m; 48H; H-4″ to H-13″ myristoyl chain), 1.70 (m; 6H; H-3″ (β protons) myristoyl chain), 2.40 (m; 6H; H-2″ (α protons) myristoyl chain), 4.38 (m; 3H; H-6′ and H-5′ ribose ring), 5.36 (m; 2H; H-3′ and H-4′ ribose ring), 5.80 (d; 1H; *J* = 8.0; H-5 uracil ring), 6.08 (d, 1H, *J* = 5.1; H-2′ ribose ring), 7.45 (d, 1H, *J* = 8.1; H-6 uracil ring). ^{13}C NMR spectroscopy data and 2D correlations as reported elsewhere.[17,19] ESI-MS (positive mode) *m/z*: Calculated for $C_{51}H_{90}N_2O_9$: 875.2683; found, 898.6 $[M+Na]^+$.

3′,4′,6′-*O*-tristearoyl uridine or uridine tri-stearate, was isolated as a white solid (20% yield), melting point: 68–69 °C. ^{1}H NMR ($CDCl_3$), δ ppm (multiplicity; integration; *J* (Hz); position): 0.87 (t; 9H; *J* = 6.5; H-18″ stearoyl chain), 1.25 (m; 48H; H-4″ to H-17″ stearoyl acid), 1.56 (m; 6H; H-3″ (β protons) stearoyl acid), 2.32 (m; 6H; 2″ (α protons) stearoyl acid), 4.35 (m; 3H; H-6′ and H-5′ ribose ring), 5.35 (m; 2H; H-3′ and H-4′ ribose ring), 5.80 (d; 1H; *J* = 8.0; H-5 uracil ring), 6.05 (d, 1H, *J* = 4.6; H-2′ uracil ring), 7.49 (d, 1H, *J* = 8.2; H-6 uracil ring). ^{13}C NMR spectroscopy data and 2D correlations as reported elsewhere.[17] ESI-MS (positive mode) *m/z*: Calculated for $C_{63}H_{114}N_2O_9$: 1043.5872; found, 1066.9 $[M+Na]^+$ and 1044.9 $[M+H]^+$.

Calorimetric analysis

The DSC analysis was performed using a DSC1 Mettler Toledo Star system equipped with a calorimetry cell and employing Mettler Star software (11.1 version). The sensitivity was automatically chosen as the maximum available in the calorimetric system. The reference was prepared with 120 μl of TRIS buffer solution (50 mM, pH = 7.4) in DSC aluminium pan (160 μl of total volume), hermetically sealed.

MLV preparation

The phospholipid and tri-acyl ester derivatives of uridine were separately dissolved in a chloroform/methanol (1 : 1, v : v) mixture. The DMPC-MLV were prepared in the absence and presence of uridine or derivatives (Figure 1) at different molar fractions (0.015, 0.03, 0.045, 0.06, 0.09, 0.12, 0.15 and 0.18) as follows: aliquots of the compound solutions were added on glass tubes (which contained 7 mg of phospholipid), in appropriates amount to have the chosen molar fraction. The solvents mixture was removed under nitrogen flow (in a water bath at 37 °C) to obtain the lipid films; then, the glass tubes with the lipid films were subjected to freeze drying to remove residual solvents. 168 μl of TRIS solution (50 mM) at pH = 7.4 were added

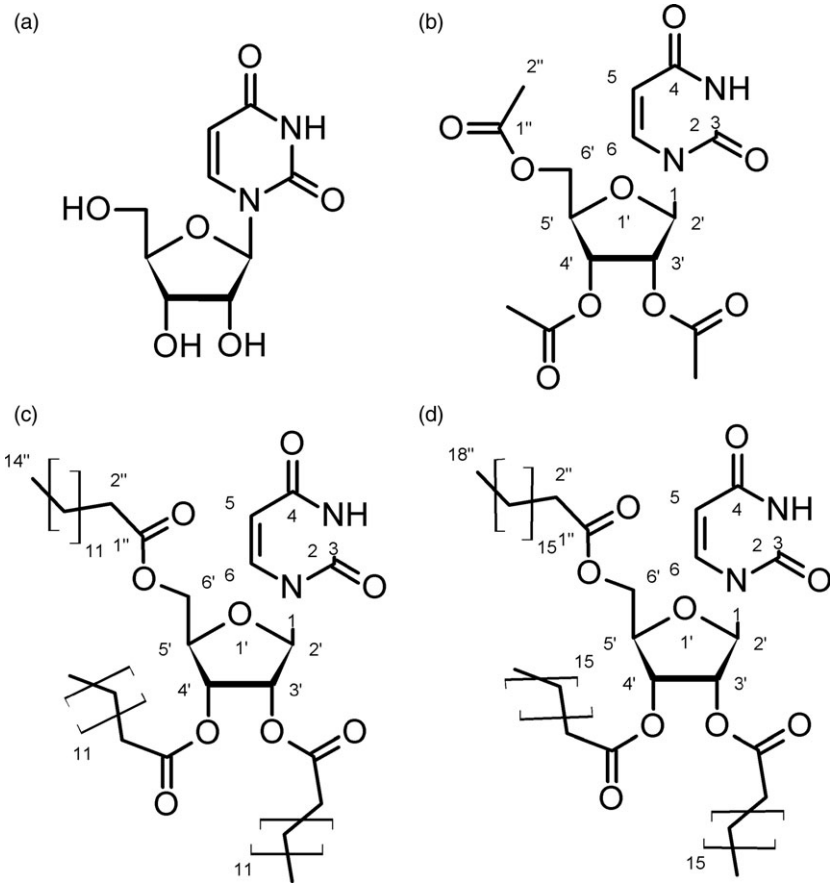

Figure 1 Chemical structure of compounds: (a) uridine; (b) triacetate uridine; (c) trimyristoyl uridine; and (d) tristearoyl uridine.

on each sample that were heated at 37 °C in a water bath and vortexed 1 min (three times) and left at 37 °C for 1 h.[7,21]

Compounds-MLV interaction studies

An aliquot of 120 µl of MLV aqueous dispersion (without or with the evaluated compounds) was put into an aluminium calorimetric pan of 160 µl that was hermetically sealed and submitted to DSC analysis. The heating scans were between 5 and 37 °C with heating rate of 2 °C/min. The cooling scans were between 37 and 5 °C with cooling rate of 4 °C. The calorimetric scans (heating-cooling) were carried out at least three times in the temperature range indicated.[26]

The third curve of the heating scans was taken for analysis and calculation of the thermotropic parameters involved in the phase transitions. Calculation models employed for the graphics are as follows: $\Delta T = T_m - T^0_m$; where T_m is the transition temperature of DMPC-MLV prepared in the presence of the substances and T^0_m is the transition temperature of pure DMPC-MLV. $\Delta\Delta H = \Delta H - \Delta H^0$, where ΔH is the enthalpy variation of MLV prepared in the presence of the substances and ΔH^0 is the enthalpy variation of pure DMPC-MLV.

Statistical analysis

Statistical analysis of data was performed using the Kruskal–Wallis and the Dunn's tests. A probability, P, of less than 0.05 was considered significant.

Results and Discussion

Chemical

The tri-acyl ester derivatives of uridine with aliphatic acid of short chain were obtained with quantitative yield in brief time thanks to the reaction for the triacetate of uridine formation carried out in acetic anhydride as solvent and reactive and the use of TEA and DMAP as nucleophilic catalysts.[17]

The Steglich method allowed the esterification of all three hydroxyl groups of the nucleoside, the products were obtained with yields about 20% for the fatty acids. The low yields were possible because this method required the O-acyl-isourea intermediate formation among the carboxylic acid and the *N,N′*-dicyclohexylcarbodiimide (coupling agent), the intermediate showed on the carbonyl group a reactivity similar to anhydride derivative. Moreover, this compound presented a bulky structure; therefore, the steric effects were higher and the effective shocks between intermediate and nucleophile (hydroxyl groups of the uridine) decreased.[16]

DSC analysis

The aim of this research was to investigate the interaction among the nucleoside-type substances (i.e. uridine and its derivatives) and the phospholipid bilayers of MLV and the miscibility between uridine derivatives and DMPC-MLV. Therefore, MLV of DMPC were prepared in the absence and presence of these compounds at different concentrations and submitted to calorimetric analysis. The calorimetric curves generated through the DSC analysis are provided according to the increase in molar fractions for the compounds evaluated. The DSC thermogram of the DMPC-MLV typically shows: a first peak around 14.5 °C corresponding to the pre-transition (Figure 2), which is related to the transition from the lamellar gel phase (in this phase the phospholipid chains are mainly distributed in trans configuration) to the rippled gel phase (in this phase the surface of phospholipidic bilayer shows a periodic rippled structure) and this behaviour is predominantly associated with changes in the interaction force on the polar phase; a second and main peak at around 25 °C (Figure 2) related to the transition from the rippled gel phase to a disordered state (called crystal liquid phase) associated mainly to the lipophilic interaction among the acyl chains of DMPC-MLV.[28,29] Therefore, the main peak transition can be employed to have information on the nature and strength of interactions in the system composed of DMPC-MLV and exogenous compounds.[20]

The DSC thermograms of DMPC-MLV in the absence and presence of uridine and its uridine triacetate at different molar fractions are shown in Figure 2a and 2b. The two compounds exerted an insignificant effect on the MLV thermogram. The pre-transition peak remains almost unchanged. The 3′,4′,6′-*O*-triacetyl uridine caused only a small broadening of the pre-transition peak with (starting from 0.045 molar fraction).

The nucleoside and the tri-acetyl ester derivative exerted just an imperceptible effect also on the main transition peak of DMPC-MLV. This behaviour suggests that the compound, due to the polar nature, presents a strong interplay with the polar phase (formed by the phosphatidylcholine head of DMPC) of phospholipid multilamellar vesicles, but does not with the lipophilic phase (formed for the acyl chain of the DMPC). Such an interaction produces a mobility and change in the acyl chain inclination of the phospholipid molecules.[21]

The triacetate uridine produces a small decrease in the enthalpy of the main transition starting from 0.06 molar fraction (Figure 3b), which indicates that this derivative presents a weaker interaction with the lipophilic phase of the phospholipidic bilayers (Figures 2b and 3b). Nevertheless, the attractive interactions between the three substituent groups of the derivative and the myristoyl chains of phospholipid are weak because the acetyl chain is very short and the lipophilic character of the tri-acetyl ester of uridine is weak. Because of this, the compound can penetrate to a small extent the lipophilic phase and interplay only with a small portion of acyl chains.[21]

From the thermograms of DMPC-MLV prepared with the tri-acyl derivatives with fatty acyl chain, it can be easily observed that these products cause a strong effect on the thermotropic behaviour of the DMPC-MLV (Figure 2c and 2d). The thermograms related to both substances are characterized by the complete disappearance of the pre-transition peak starting from lowest molar fraction (0.015) implicating that these compounds strongly affect the polar phase of the MLV phospholipids; thereby, causing changes on the tilt of phospholipid molecules and the configuration of the acyl chains (Figure 2). Consequently, trimyristoyl and tristearoyl uridine present a strong interaction with the phosphatidylcholine head of DMPC-MLV, being possible only if the nucleoside part, particularly the uracil ring, is preferably localized or oriented towards the polar phase.[20]

The thermogram of MLV with trimyristoyl uridine suggests a strong effect of this compound on the thermotropic behaviour of the DMPC-MLV (Figure 2c). The compound causes a visible shift of the main transition peak toward lower temperature beginning from the smallest concentration employed. The effect of the derivative implies that some moiety of the molecule is incorporated in the lipophilic phase of the bilayers, but this part should be of lipophilic nature; therefore, the myristoyl chains of the tri-acyl ester derivative are embedded between the acyl chains of the phospholipid bilayers. In addition, this substance, as its concentration increases, produces a gradual broadening of the main peak and decreasing of the enthalpy associated with this, starting from the lowest molar fraction used until the nearly complete disappearance of transition peak at the highest concentration employed (Figure 3b). This means that the aliphatic chains of derivative strongly interact with the acyl chains of the DMPC-MLV; this interaction

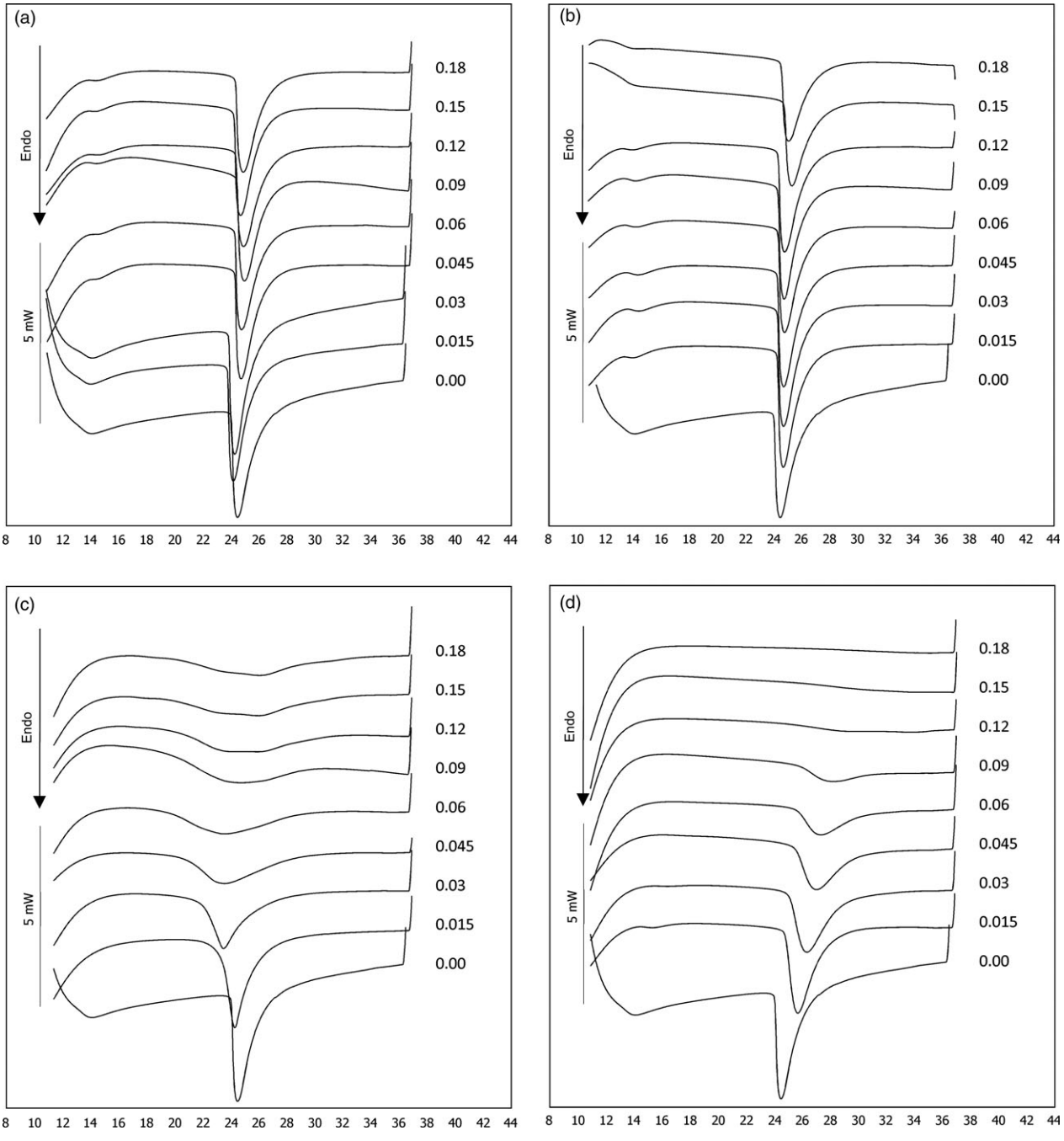

Figure 2 Differential scanning calorimetry thermograms, in heating mode, of L-α-dimyristoylphosphatidylcholine-multilamellar vesicles prepared in the presence of increasing molar fractions of uridine (a), uridine triacetate (b), trimyristoyl-uridine (c) and tristearoyl-uridine (d). The X axes report the temperature (°C).

prevents the cooperativity between the acyl chains of phospholipid molecules in the transition from gel to liquid crystalline, thus causing enlargement of the peak. Moreover, starting from the molar fraction 0.09, a second signal appears. The temperature of this signal (at lower temperature) remains almost unchanged but its intensity decreases in favour of the peak at higher temperature. This can be due to a not uniform distribution of the derivative inside the phospholipid bilayers and, consequently, to the presence of two different phospholipid regions: one reach in the compound and the other poor in the same.[30,31] These results are in good agreement with those obtained in a

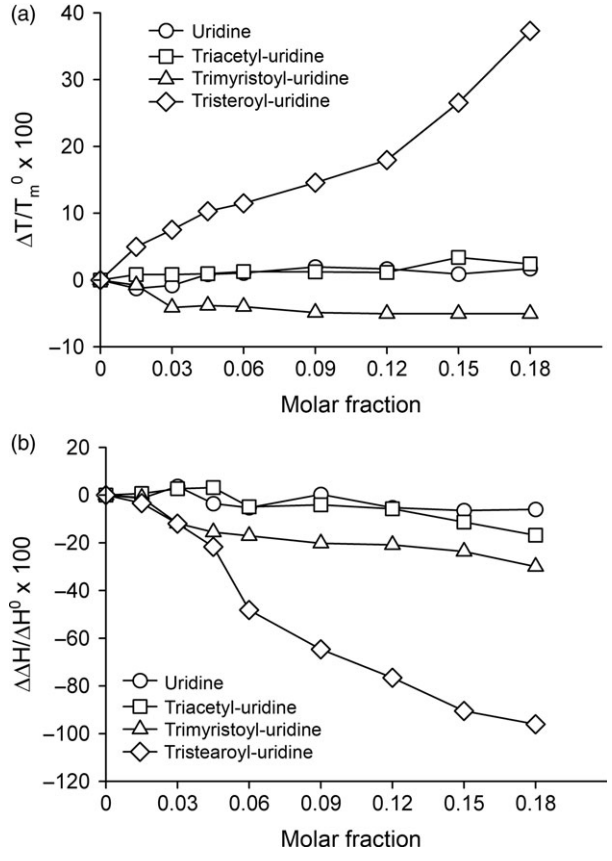

Figure 3 (a) Transition temperature variation, as $\Delta T/T^0{}_m$, of L-α-dimyristoylphosphatidylcholine-multilamellar vesicles, as a function of the molar fraction of compounds evaluated. (b) enthalpy variation, as $\Delta\Delta H/\Delta H^0$, of L-α-dimyristoylphosphatidylcholine-multilamellar vesicles prepared in the presence of the molar fraction of compounds evaluated.

recent study on the interaction between uridine-trimyristate and DPPC and DPPS phospholipid monolayers as non-tumorogenic and tumorogenic biomembrane cell models, respectively, prepared using the Langmuir-Blodgett technique.[23]

The tri-acyl ester derivative of uridine with stearic acid presented a strong effect on the thermotropic behaviour of DMPC bilayers. In the thermograms, it is easily observed a great shift of the main transition peak toward higher temperature and a strong broadening, starting from the smallest concentration used. The great effect caused by tristearoyl uridine indicates a strong interaction of the lipophilic part of compound with the lipophilic part of DMPC. Also, the enthalpy associated with the transition from the gel to the liquid crystalline phase decreased with increasing tristearoyl uridine molar fractions (Figures 2b and 3b). These results are due to the strong interaction among the lipophilic chains (belonging to fatty acid) of the derivative and the acyl chains of the phospholipids of the bilayers, which induces a drastic decrease in the cooperativity presents between the acyl chain of phospholipid molecules in the MLV.

The shift of the main transition peak towards higher temperature shown for the tristearoyl uridine indicates an unusual thermotropic behaviour of DMPC-MLV when the exogenous substance interacts with the centre of the phospholipid and seems to stabilize this; therefore, the temperature of the main transition is higher. This is possible if the system formed by the lipophilic chains of derivative and acyl chains of phospholipid are interdigitated forming a complex structure.[27] The DSC thermograms (Figure 2b) for the tristearoyl uridine did not show the appearance of a second peak into the main transition, as observed in the calorimetry curves related to tri-myristoyl uridine (Figure 2a). This is possibly due to the fact that tristearoyl uridine uniformly distributed in the multilamellar vesicles (rich and poor regions of the compound inside the phospholipidic bilayers did not form). Moreover, this derivative causes a significant decrease in the enthalpy associated with main transition with the increase in the amount of the compound employed (Figure 2b), until the complete disappearance of the transition peak at the highest molar fraction employed in this work. The correlation between the effect exerted by the tested derivatives on the biomembrane model, and their characteristics clearly indicate an improved incorporation efficiency of the derivatives with respect to uridine into the biomembrane model; with tristearoyl uridine and trimyristoyl uridine having the highest incorporation efficiency, probably due to their stronger lipophilic character.

The temperature transition and enthalpy variation values were analysed using the Kruskal–Wallis test. The test confirmed the results significance with $P < 0.05$. The data were also analysed using the Dunn's test. Regarding the transition temperature results, the test indicated as of high significance the differences between uridine and tristearoyl uridine, triacetyl uridine and trimyristoyl uridine, trimyristoyl uridine and tristearoyl uridine. With regard to the enthalpy variation values, the Dunn's test indicated that the differences between uridine and tristearoyl uridine and between triacetyl uridine and tristearoyl uridine are highly significant.

Conclusion

The lipophilic character acquired by the tri-acyl ester derivatives of uridine nucleoside was possible to be achieved through the conjugation reactions with aliphatic acid of long chain on the all hydroxyl groups of ribose. The aliphatic chains of fatty acid linked to the nucleoside through ester bonds produced an affinity with the lipophilic phase of DMPC-MLV used as biomembrane

model. The effect of uridine and tri-acetate uridine on the thermotropic behaviour of DMPC-MLV was the one expected for these compounds that indicates their interaction with the phospholipid polar head and their location within the polar phase of the multilamellar vesicles. The effect of trimyristoyl uridine and tristearoyl uridine on DMPC-MLV indicates that a strong interaction of the compounds and the lipophilic part of DMPC occurs. The extent of the interaction depends on the length of the alkyl chain.

The obtained results highlight the usefulness of the DSC technique used in this study in helping to understand uridine derivatives insertion and interaction in/with lipid assemblies such as those of biological membrane; moreover, to study the performance of different molecules with biomembrane model, in view of possible future *in vivo* studies.

Declarations

Conflict of interests

The authors state that there is no conflict of interests in this work.

Funding

Authors thank financing to the University of Antioquia (Project CODI CIQF-155 2012–2014, Strategy of Sustainability 2015–2016).

Reference

1. Wagner CR *et al.* Pronucleotides: toward the in vivo delivery of antiviral and anticancer nucleotides. *Med Rev Res* 2000; 20: 417–451.
2. Zhenchuk A *et al.* Mechanisms of anti-cancer action and pharmacology of clofarabine. *Biochem Pharmacol* 2009; 78: 1351–1359.
3. Mehta DR *et al.* Fludarabine and cytarabine in patients with acute myeloid leukemia refractory to two different courses of front-line chemotherapy. *Leuk Res* 2011; 35: 885–888.
4. Moysan E *et al.* Gemcitabine versus modified gemcitabine: a review of several promising chemical modification. *Mol Pharm* 2013; 10: 430–444.
5. Shelton J *et al.* Metabolism, biochemical actions, and chemical synthesis of anticancer nucleosides, nucleotides, and base analogs. *Chem Rev* 2016; 116: 14379–14455.
6. Gorzkiewicz M *et al.* Dendrimers as nanocarriers for nucleoside analogues. *Eur J Pharm Biopharm* 2017; 114: 43–56.
7. Sarpietro MG *et al.* Synthesis of n-squalenoyl cytarabine and evaluation of its affinity with phospholipid bilayers and monolayers. *Int J Pharm* 2011; 406: 69–77.
8. Gao L-J *et al.* l-Aspartic and l-glutamic acid ester-based ProTides of anticancer nucleosides: synthesis and antitumoral evaluation. *Bioorg Med Chem Lett* 2016; 26: 2142–2146.
9. Castelli F *et al.* Enhancement of gemcitabine affinity for biomembranes by conjugation with squalene: differential scanning calorimetry and Langmuir-Blodgett studies using biomembrane models. *J Colloid Interface Sci* 2007; 316: 43–52.
10. Radi M *et al.* In vitro optimization of non-small cell lung cancer activity with troxacitabine. *J Med Chem* 2007; 50: 2249–2253.
11. Chhikara BS *et al.* Synthesis and evaluation of fatty acyl ester derivatives of cytarabine as anti-leukemia agents. *Eur J Med Chem* 2010; 45: 4601–4608.
12. Valade A *et al.* Target-assisted selection of galactosyltransferase binders from dynamic combinatorial libraries. An unexpected solution with restricted amounts of the enzyme. *ChemBioChem* 2006;7:1023–1027.
13. Wandzik I *et al.* Synthesis of 2-deoxy-hexopyranosyl derivatives of uridine as donor substrate analogues for glycosyltransferases. *Bioorg Chem* 2009; 37: 211–216.
14. Yamamoto T *et al.* Biochemistry of uridine in plasma. *Clin Chim Acta* 2011; 412: 1712–1724.
15. Hrdlicka PJ *et al.* Synthesis and biological evaluation of nucleobase-modified analogs of the anticancer compounds 3′-C-ethynyluridine (Eurd) and 3′-C-ethynylcytidine (Ecyd). *Med Chem* 2005; 13: 1249–1260.
16. Berrío Escobar JF *et al.* Synthesis and cytotoxic activity of tri-acyl ester derivatives of uridine in breast cancer cells. *Ars Pharm* 2016; 57: 183–191.
17. Berrío Escobar JF *et al.* Synthesis and cytotoxic activity of per-acetylated and halogenated derivatives of nucleosides in breast cancer cells. *Ars Pharm* 2017; 58: 145–154.
18. McEvillyn M *et al.* Use of uridine triacetate for the management of fluorouracil overdose. *Am J Health-Syst Pharm* 2011; 68: 1806–1809.
19. Bach D *et al.* Phospholipid/cholesterol model membranes: formation of cholesterol crystallites. *Biochim Biophys Acta – Biomembr* 2003; 1610: 187–197.
20. Raudino A *et al.* Differential scanning calorimetry (DSC): theoretical fundamentals. In: Pignatello R, ed. *Drug-Biomembrane Interaction Studies: The Application of Calorimetric Techniques*. Cambridge, UK: Woodhead Publ., 2013: 127–168.
21. Sarpietro MG *et al.* Interaction of α-hexylcinnamaldehyde with a biomembrane model: a possible MDR reversal mechanism. *J Nat Prod* 2015; 78: 1154–1159.
22. Alves AC *et al.* Biophysics in cancer: the relevance of drug-membrane interaction studies. *Biochim Biophys*

Acta – Biomembr 2016; 1858: 2231–2244.
23. Grosso Salis LF *et al.* Interaction of 3′,4′,6′-trimyristoyl-uridine derivative as potential anticancer drug with phospholipids of tumorigenic and non-tumorigenic cells. *Appl Surf Sci* 2017; 426: 77–86.
24. Rodrigues JC *et al.* Incorporation of bacitracin in Langmuir films of phospholipids at the air-water interface. *Thin Solid Films* 2017; 622: 95–103.
25. Diociaiuti M *et al.* Monosialoganglioside-GM1 triggers binding of the amyloid-protein salmon calcitonin to a Langmuir membrane model mimicking the occurrence of lipid-rafts. *Biochem Biophys Reports* 2016; 8: 365–375.
26. Librando V *et al.* Calorimetric evidence of interaction of brominated flame retardants with membrane model. *Environ Toxicol Pharm* 2015; 39: 1154–1160.
27. Tapia MJ *et al.* β-phase formation of poly (9,9-dioctylfluorene) induced by liposome phospholipid bilayers. *J Phys Chem B* 2011; 115: 5794–5800.
28. Koynova R, Caffrey M. Phases and phase transitions of thephosphatidylcholines. *Biochim Biophys Acta* 1998; 1376: 91–145.
29. Walde P. Preparation of vesicles (liposomes). In: Nalwa HS, ed. *ASP Encyclopedia of Nanoscience and Nanotechnology*. Stevenson Ranch, CA: American Scientific Publishers, 2004: 43–79.
30. Polikandritou Lambros M *et al.* Effects of cyclosporin A on model lipid membranes. *Chem Phys Lipids* 2004; 131: 63–69.
31. Alves ID *et al.* Membrane interaction and perturbation mechanisms induced by two cationic cell penetrating peptides with distinct charge distribution. *Biochim Biophys Acta* 2008; 1780: 948–959.