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Conjugation of squalene to acyclovir improves the affinity for biomembrane models

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ABSTRACT

Differential scanning calorimetry was used to study the interaction of acyclovir and its prodrug squalenoyl–acyclovir (obtained by conjugation of 1,1',2-tris-nor-squalene acid (squaleneCOOH) with acyclovir) with biomembrane models made of DMPC multilamellar vesicles with the aim to verify whether a stronger interaction of the prodrug with respect to the free drug can be obtained. Multilamellar vesicles were prepared in the presence of increasing molar fractions of acyclovir, squaleneCOOH or prodrug and the effect of the compounds on the thermotropic behavior of vesicles was researched, revealing no effect of acyclovir but a strong effect of squaleneCOOH and prodrug. To evaluate if acyclovir, squaleneCOOH and prodrug can be absorbed by the biomembrane model, an experiment was carried out in which the considered compounds were left in contact with the biomembrane model and their eventual uptake was evaluated analyzing the effect on the thermotropic behavior of the biomembrane model. A very small uptake was revealed for all the compounds. To check the potential use of liposomes as a delivery system for the prodrug, the biomembrane models were incubated with liposomes loaded with the compounds and the compounds transferring from the loaded liposomes to the unloaded biomembrane model was followed. The results suggest that liposomes could be used to deliver the squalenoyl–acyclovir to the biomembrane model.

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1. Introduction

Acyclovir [9-(2-hydroxyethoxymethyl)guanine; ACV], an acyclic nucleoside analogue, has shown a potent antiviral activity. It is known to inhibit the replication of herpes viruses including herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus, and Epstein–Barr virus in cell cultures and in animals (Biron and Elion, 1980; O'Brien and Campoli-Richards, 1989; Li et al., 2006).

Unfortunately, acyclovir has an oral bioavailability of only 20% because of its low intestinal permeability (de Miranda and Blum, 1983). The poor aqueous solubility and lipophilicity of acyclovir is a limitation to its pharmaceutical application. In order to overcome these limitations, a number of ACV prodrugs were synthesized. Valacyclovir, the L-valine ester prodrug of acyclovir, was synthesized showing an oral bioavailability of 54% (Soul-Lawton et al., 1995). Dipeptide prodrugs of ACV were synthesized with the aim to improve the ocular bioavailability of ACV after topical instillation of an aqueous solution of the drug; the ACV prodrugs

showed to be stable, soluble and to possess in vivo antiviral efficacy (Anand et al., 2003). Giammona et al. (1995) modified acyclovir by acylation of the hydroxyl group in the side chain with succinic anhydride and the O-succinylacyclovir derivative obtained was coupled to α,β -poly(N-2-hydroxyethyl)-DL-aspartamide to give a conjugate which showed a higher bioavailability after oral and intravenous administration with respect to the free acyclovir.

The enhancement of the lipophilic character of drugs was related to their increased interaction with biomembrane models and then it can be used to improve their ability to enter the cells (Pignatello et al., 2006) allowing to obtain useful information in the correlation between the effect on the biomembrane models and the bioavailability. Recently, the acyclic isoprenoid chain of squalene was coupled to gemcitabine to obtain the lipophilic prodrug squalenoyl–gemcitabine whose interaction with biomembrane models was studied, giving indication of the improved interaction of the prodrug with respect to the free gemcitabine (Castelli et al., 2007).

In this paper, the interaction of the prodrug squalenoyl–acyclovir (SQACV) (obtained by conjugation of 1,1',2-tris-nor-squalene acid (squaleneCOOH) with acyclovir) (Fig. 1) with biomembrane models was studied with the aim to verify if a different interaction of the prodrug with respect to the free drug could

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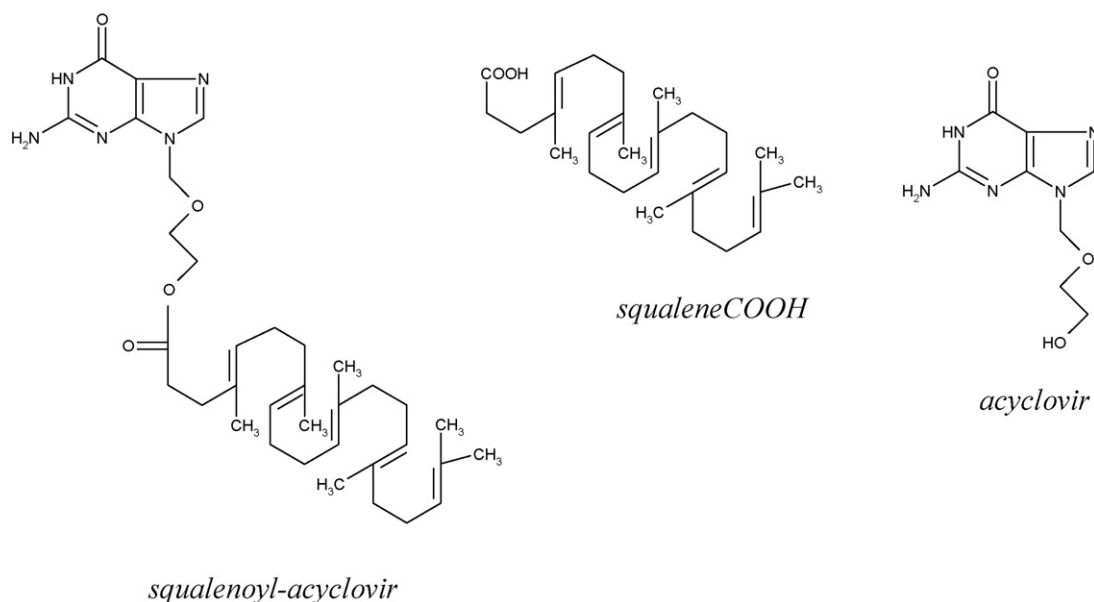


Fig. 1. Squalenoyl-acyclovir, squaleneCOOH and acyclovir structure.

be obtained. Experiments were carried out to evaluate the ability of the prodrug to be absorbed by the biomembrane models, and to check the potential use of liposomes as a delivery system for the prodrug.

Multilamellar vesicles (MLV) made of dimyristoylphosphatidylcholine (DMPC), used as biomembrane models, upon heating, undergo a phase transition from an ordered gel-like structure to a disordered fluid-like structure at a well defined temperature (T_m) and with a defined enthalpy variation (ΔH). This phase transition is strongly affected by the presence of foreign molecules dissolved in the phospholipid bilayer (Mouritsen and Bloom, 1984; Jorgensen et al., 1991). The differential scanning calorimetry (DSC), which can detect the phase transition of the MLV, was used (Mabrey-Gaud, 1981; Bach, 1994; Huang and Li, 1999; Castelli et al., 2005, 2008).

The results can give information on the localization of the examined compounds inside the biomembrane and on the possible use of liposomes as a suitable way to favour the transport of the prodrug into the biomembranes.

2. Materials and methods

2.1. Materials

Acyclovir (purity $\geq 99\%$) and squalene (purity=98%) were purchased from Sigma–Aldrich (Italy). 1,1',2'-*tris-nor*-squalene aldehyde was obtained as previously reported (Ceruti et al., 2005). SQACV was obtained following the general method previously described (Stella et al., 2005) by reaction of 1,1',2'-*tris-nor*-squalene acid with acyclovir. It was completely pure, as revealed by ^1H NMR and mass analysis. Concerning the possibility of obtaining either an ester or an amide linkage between the squalenoyl chain and acyclovir, ^1H NMR analysis revealed the presence of the free amino group at 6.57δ (2 H, s, NH_2), while the signal at about 12δ of the hypothetical amidic group was completely absent. It was also confirmed by comparison of ^1H NMR spectra of ester and amide acetyl derivatives of acyclovir reported in the literature (Matsumoto et al., 1987). ^1H NMR (DMSO): δ , 1.52–1.65 (18 H, m, allylic CH_3), 1.94–2.10 (16 H, m, allylic CH_2), 2.13 (2 H, t, $\text{OCOCH}_2\text{CH}_2$), 2.31 (2 H, t, OCOCH_2), 3.63 (2 H, m, 3'- OCH_2), 4.06 (2 H, m, 4'- CH_2OCO), 5.03–5.25 (5 H, m, vinylic CH), 5.32 (2 H, s, 1'- NCH_2O), 6.57 (2 H, s,

NH_2), 7.78 (1 H, s, 8-CH), 10.69 (1 H, s, cycle 1-NHCO). MS (CI): m/z 608 (M^+ , 100).

Synthetic L- α -dimyristoylphosphatidylcholine (DMPC) (purity=99%) was obtained from Genzyme (Switzerland). Lipids were chromatographically pure as assessed by two-dimensional thin layer chromatography. Lipid concentration was determined by the phosphorous analysis (Rouser et al., 1970). 50 mM Tris(hydroxymethyl)-aminomethane (Tris) adjusted at pH 7.4 with hydrochloric acid was used.

2.2. Differential scanning calorimetry

DSC was performed by using a Mettler TA Star^e equipped with a DSC 822^e calorimetric cell and a Mettler STAR^e V 8.10 Software. The reference pan was filled with Tris solution.

The sensitivity was automatically chosen as the maximum possible by the calorimetric system. The calorimetric system was calibrated, in transition temperature and enthalpy changes, by using indium, stearic acid and cyclohexane by following the procedure of the Mettler TA STAR^e Software.

2.3. Compounds/MLV interaction

To study the interaction between the compounds and the biomembrane model, MLV were prepared in the presence and absence of increasing concentrations (0.00, 0.015, 0.03, 0.045, 0.06, 0.09, 0.12 and 0.15 molar fraction with respect the phospholipid) of the studied compounds with two different procedures depending on the compound. With regard to squaleneCOOH and SQACV, stock solutions of DMPC (17.95 mg/ml), squaleneCOOH (2.98 mg/ml) and SQACV (3.12 mg/ml) in chloroform were prepared and used straight after. DMPC solution aliquots were put into glass tubes, in order to have the same amount of lipid (7 mg), where aliquots of compound solution were added to obtain increasing molar fractions with respect to the lipid. The solvent was removed under nitrogen flow and the resulting films were freeze-dried under vacuum. MLV were obtained adding 168 μl of Tris solution, heating at 37°C for 1 min and shaking for 1 min, for three times. Afterward, MLV were stored, 1 h, at 37°C . As far as regard acyclovir, after the DMPC films were freeze-dried, an exact amount of acyclovir solution in 50 mM

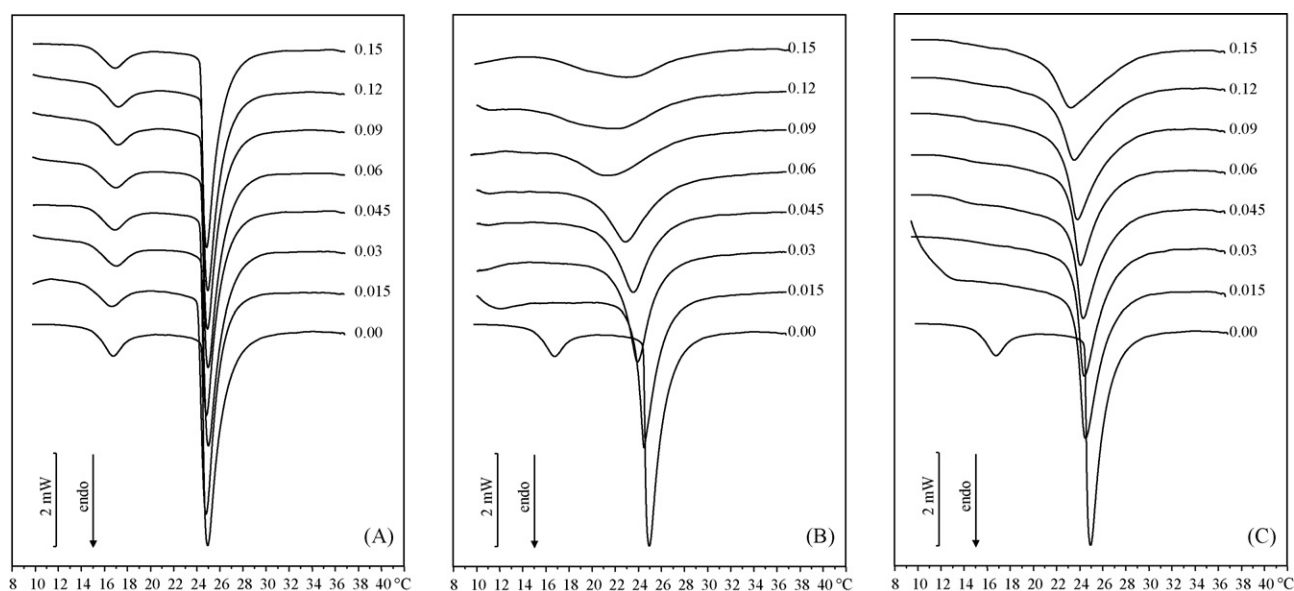


Fig. 2. Calorimetric curves, in heating mode, of DMPC MLV prepared in the presence of (A) acyclovir, (B) squaleneCOOH and (C) squalenoyl–acyclovir at increasing molar fractions.

Tris (pH = 7.4), in order to have the considered molar fraction, was added.

Then, aliquots of 120 μ l (5 mg of lipid) of MLV aqueous dispersion were transferred into a 160 μ l DSC aluminum pan, hermetically sealed, and submitted to DSC analysis as follows: a heating scan from 5 to 37 $^{\circ}$ C at the rate of 2 $^{\circ}$ C/min; a cooling scan from 37 to 5 $^{\circ}$ C at the rate of 4 $^{\circ}$ C/min; for at least three times. To check the reproducibility of the results, the experiments were repeated in triplicate; aliquots of all samples were then extracted from the calorimetric aluminum pans and used to determine, by the phosphorous assay (Rouser et al., 1970), the exact amount of phospholipids present in each sample.

2.4. Permeation experiments

To study the ability of the examined compounds to go through the aqueous medium and permeate the biomembrane model, the following experiment was carried out: DMPC MLV aqueous dispersion was left in contact with a fixed amount of chemical (powdered: acyclovir; oily: squaleneCOOH and SQACV) (to obtain a 0.12 molar fraction with respect to the phospholipid vesicles aqueous dispersion) placed in the bottom of the DSC pan. The pan was hermetically sealed and then submitted to subsequent calorimetric cycles according to the following procedure:

- (1) a scan from 5 to 37 $^{\circ}$ C, at the rate of 2 $^{\circ}$ C/min, to detect the interaction between the compound (which eventually dissolves in the aqueous medium and transfers to phospholipid membrane) and the biomembrane model during the heating of the sample;
- (2) 1 h, at 37 $^{\circ}$ C, to permit the compound to permeate the phospholipid bilayers which, at this temperature, are in a disordered state, the temperature being over the transition temperature of the phospholipids employed;
- (3) a cooling scan between 37 and 5 $^{\circ}$ C, at the rate of 4 $^{\circ}$ C/min, to go back to the starting step.

This procedure was run at least eight times to detect variations in the temperature of the calorimetric peak (due to the uptake process) occurring mainly during the period when the temperature is over the transition temperature.

2.5. Lipophilic carrier/biomembrane model transfer experiments

These experiments were carried out to evaluate the ability of the compounds to transfer from a liposomal carrier (DMPC MLV loaded with a determined amount of each compound) to the biomembrane model (unloaded DMPC MLV). For this purpose, 60 μ l of DMPC MLV loaded with a 0.12 molar fraction of compound was put into a 160 μ l DSC pan and then 60 μ l of unloaded DMPC MLV aqueous dispersion was added. The samples were hermetically sealed in the pan and submitted to subsequent calorimetric cycles following the same step procedure reported in the previous section.

3. Results and discussion

3.1. Compounds/MLV interaction

In order to improve the interaction of acyclovir with biomembrane models a prodrug was obtained by the conjugation of squaleneCOOH to acyclovir. The interaction of the prodrug and its precursors (squaleneCOOH and acyclovir) with biomembrane model was studied. DMPC MLV were prepared in the presence of increasing molar fractions of compounds and submitted to DSC analysis. The recorded calorimetric curves were compared with that of pure DMPC MLV (Fig. 2A–C). The calorimetric curve of pure DMPC MLV presents, as known, a small peak (at about 17 $^{\circ}$ C), called the pretransition peak, associated to the tilting of the phospholipid lipophilic chains, and a main peak (at about 25 $^{\circ}$ C) which arises from the gel–liquid crystalline (L_{β} – L_{α}) phase transition (Tenchov and Koynova, 1996; Marsh, 1996; Walde, 2004). The variation of the thermotropic parameters of the calorimetric curve indicates that the compounds interact with the biomembrane models. The calorimetric curves of the MLV prepared in the presence of increasing molar fractions of acyclovir (Fig. 2A) do not show significant variations with respect to that of DMPC MLV; it means that the acyclovir does not interact with the phospholipid bilayers, but, due to its mainly hydrophilic nature (Majumdar et al., 2009), distributes in the MLV aqueous layers or in the bulk phase. The calorimetric curves in the presence of squaleneCOOH (Fig. 2B) show that the pretransition peak disappears at the lowest molar fraction of squaleneCOOH present, whereas the main peak is shifted toward lower temperature and broadens, giving indication of the strong

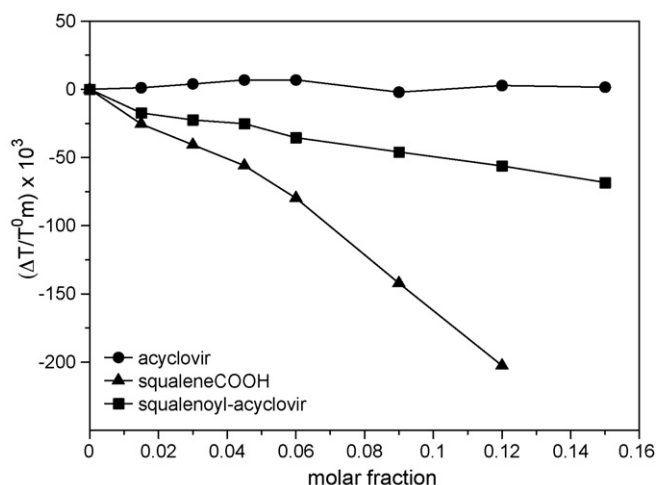


Fig. 3. Transition temperature, as $\Delta T/T_m^0$, of DMPC MLV prepared in the presence of acyclovir, squaleneCOOH and squalenoyl-acyclovir at increasing molar fraction as a function of compound molar fraction. $\Delta T = T_m - T_m^0$, where T_m is the transition temperature of DMPC MLV prepared in the presence of compound and T_m^0 is the transition temperature of pure DMPC MLV.

interaction of this compound with the MLV phospholipid bilayers. Moreover, a phase separation is strongly evident starting from 0.09 molar fraction, which could be due to a not uniform distribution of squaleneCOOH among the phospholipid molecules and, hence, to the formation of squaleneCOOH rich and squaleneCOOH poor regions (Estep et al., 1978; Bruggemann and Melchior, 1983). The calorimetric curves of DMPC MLV prepared in the presence of SQACV are shown in Fig. 2C. SQACV causes the pretransition peak to completely disappear at all the used molar fractions and the main peak to shift and broaden as its molar fraction increases. This is an evidence of the occurring interaction between SQACV and DMPC MLV. Differently from squaleneCOOH, SQACV does not cause phase separation, suggesting that this compound uniformly distributes among phospholipid molecules. This is in agreement with the results obtained by Mavromoustakos et al. (1996) which studied the effect of cannabinoids on phospholipid bilayers and observed that the more hydrophilic the molecule, the lesser its tendency to produce domains.

A comparison of the effect of SQACV with respect to those of its precursors on the biomembrane model can be obtained plotting the T_m , the ΔH and the peak width (width at peak half height, $\Delta T_{1/2}$) as a function of the compounds molar fraction present in the MLV aqueous dispersion. In Fig. 3, the T_m is plotted as $\Delta T/T_m^0$ ($\Delta T = T_m - T_m^0$, T_m being the transition temperature of DMPC MLV prepared in the presence of compound and T_m^0 being the transition temperature of pure DMPC MLV). Acyclovir causes just small T_m variation, SQACV, and even more, squaleneCOOH cause the T_m decrease. The decrease of the T_m reflects the solubility of the molecule in the fluid phase rather than in the gel phase. In fact, molecules with kinks are expected to pack easier in the fluid membrane with the disordered aliphatic chains of the lipids than in the gel state with the straight ordered lipid acyl chains differently from molecules with straight chains, like stearic acid, which interact more favourably with the gel phase of the lipid bilayer (Ortiz and Gomez-Fernandez, 1987; Peters et al., 2009). The ΔH values, as $\Delta \Delta H/\Delta H^0$ ($\Delta \Delta H = \Delta H - \Delta H^0$, with ΔH being the enthalpy variation of DMPC MLV prepared in the presence of compound and ΔH^0 being the enthalpy variation of pure DMPC MLV), are shown in Fig. 4. Acyclovir does not affect the ΔH , SQACV, and even more, squaleneCOOH lead a ΔH depression. The $\Delta T_{1/2}$ values (Fig. 5) reveal strong differences among the compounds; in fact, while acyclovir seems not to perturb the $\Delta T_{1/2}$, squaleneCOOH and

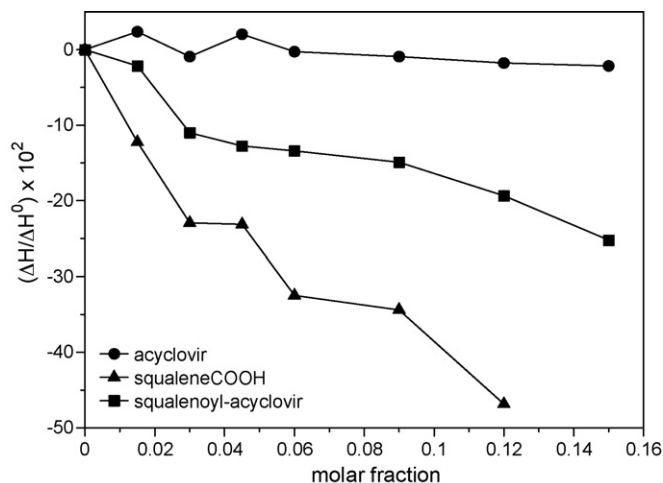


Fig. 4. Enthalpy variation, as $\Delta \Delta H/\Delta H^0$, of DMPC MLV prepared in the presence of acyclovir, squaleneCOOH and squalenoyl-acyclovir at increasing molar fraction as a function of compound molar fraction. $\Delta \Delta H = \Delta H - \Delta H^0$, where ΔH is the enthalpy variation of DMPC MLV prepared in the presence of compounds and ΔH^0 is the enthalpy variation of pure DMPC MLV.

SQACV produce the $\Delta T_{1/2}$ increase. All these results indicate that squaleneCOOH and SQACV have a fluidizing effect on the phospholipid bilayer, interact with the hydrophobic acyl chains and cause a reduction of the cooperativity of the C1–C8 region of the bilayer (Jain and Wu, 1977; Raudino and Castelli, 1998; Huang and Li, 1999; Wolka et al., 2004). And then, while acyclovir does not insert in the phospholipid bilayers, squaleneCOOH and SQACV localize in the bilayers. The different effect exerted by squaleneCOOH and SQACV on the transition phase can give information on their localization in the phospholipid bilayers. The stronger effect exerted by squaleneCOOH suggests that it enters into the bilayers more deeply than SQACV does. SqualeneCOOH could localize in a way that the polar part is next the phospholipid polar head and the remaining part parallel to the phospholipid apolar tails. This conjecture is not in contrast with the apparent length of squaleneCOOH that in its extended form is as large as 29 Å (Simon et al., 1977). Such a length is far longer than that of common phospholipids like DPPC and DMPC which form bilayers of about 20 Å per single monolayer (Nagle and Tristram-Nagle, 2000). Indeed, it is well-known that the most famous squalene-derivative, the cholesterol, easily

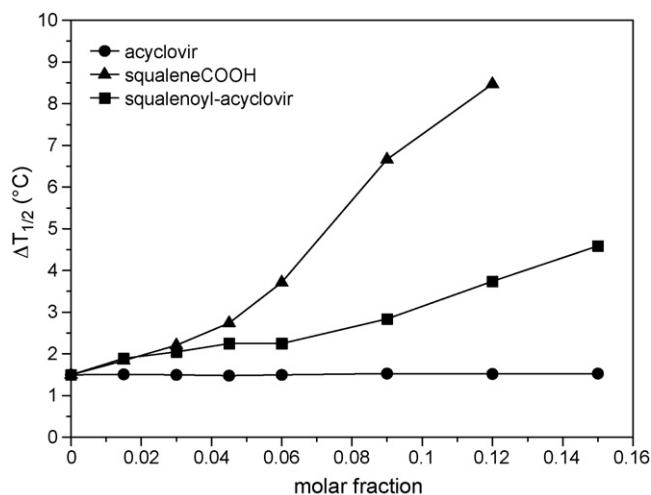


Fig. 5. Peak width (width at half height peak, $\Delta T_{1/2}$) of DMPC MLV prepared in the presence of acyclovir, squaleneCOOH and squalenoyl-acyclovir at increasing molar fraction as a function of compound molar fraction.

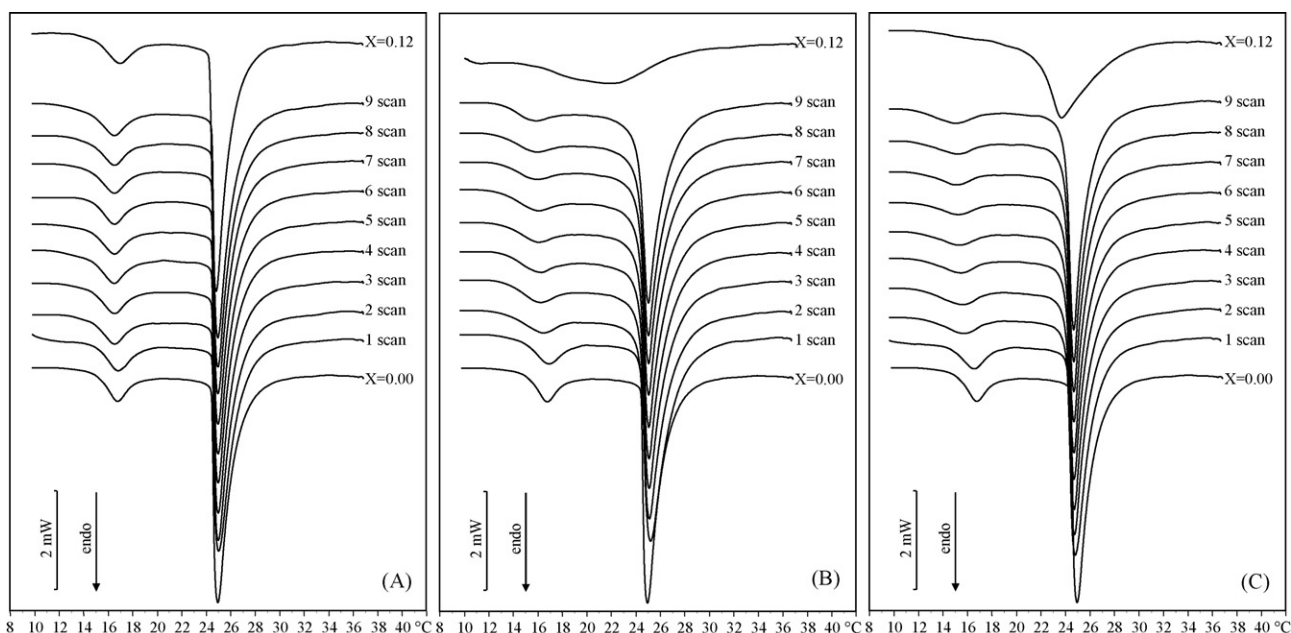


Fig. 6. Calorimetric curves, in heating mode, of DMPC MLV left in contact with (A) acyclovir, (B) squaleneCOOH and (C) squalenoyl-acyclovir at 0.12 molar fraction, at different scans (increasing incubation periods). Curve $X = 0.12$ belongs to DMPC MLV prepared in the presence of compounds at 0.12 molar fraction and is the curve which should be obtained if the compound was completely absorbed by the MLV.

accommodates into lipid bilayers of different thickness. Thus, we may suggest three different explanations for the squaleneCOOH accommodation in the lipid bilayer: (a) the hydrophobic backbone of squalene assumes a coiled conformation similar to that of its condensed derivative, the cholesterol. In this configuration, the polar head of squaleneCOOH is in contact with water while its tail basically is parallel to those of surrounding lipids. Obviously, squaleneCOOH must pay a small entropy cost in order to adopt a particular rigid conformation. (b) Some additional space to accommodate the long molecules may be gained if squaleneCOOH assumes a tilted conformation with respect to the membrane plane. Recent Molecular Dynamics simulations have confirmed such a possibility, evidencing very tilted cholesterol configuration inside the lipid bilayer (Kucerka et al., 2008). (c) Foreign molecules may change the order parameter of the surrounding lipids and, consequently, the whole thickness of the lipid bilayer. Such a strategy is adopted by cholesterol that can be easily accommodated into bilayers of different thickness: bilayers made up of long lipids become thinner (McIntosh, 1978; Gallova et al., 2004) while those constituted by short saturated (McIntosh, 1978) or unsaturated lipids (Kucerka et al., 2007) become thicker. In SQACV, the squalenoyl moiety could stay among the phospholipid tails, whereas the acyclovir moiety could protrude in the aqueous environment with a small contribution to the prodrug effect on the phospholipid thermotropic behavior.

3.2. Permeation experiments

Once the interaction of the compounds with the biomembrane model was studied, the absorption of the prodrug by the biomembrane models was evaluated; for this reason we performed experiments in which the compound (at 0.12 molar fraction) and the MLV were incubated at 37 °C and the interaction compound/MLV was monitored as soon as before the incubation and at intervals of 1 h; for completeness, experiments with neat acyclovir and squaleneCOOH were also performed. If the compound, left in the same aqueous environment of the MLV, was absorbed by them, then, a gradual modification of calorimetric curves should result and if the absorption was complete a curve similar (in shape

and thermodynamic parameters) to that obtained from the analysis of the MLV prepared in the presence of the compound at 0.12 molar fraction should be reached. The obtained calorimetric curves were, then, compared with that of the pure DMPC MLV and that of the MLV prepared in the presence of a 0.12 molar fraction of compound and are shown in Fig. 6A–C. As expected, acyclovir does not cause modification in the calorimetric curves. Squalene and SQACV cause just a negligible effect on both the pretransition peak and the main peak. The results are reported, as $\Delta T/T_m^0$, as a function of the calorimetric scans and shown in Fig. 7. Acyclovir does not cause any transition temperature variation; then no interaction between acyclovir and MLV occurs. SqualeneCOOH and SQACV cause a negligible decrease of the transition tempera-

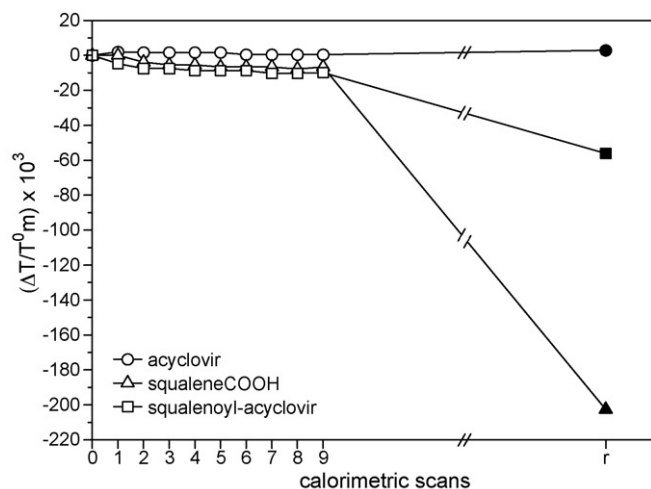


Fig. 7. Transition temperature, as $\Delta T/T_m^0$, of DMPC MLV left in contact with acyclovir, squaleneCOOH and squalenoyl-acyclovir at 0.12 molar fraction, as a function of the calorimetric scans. $\Delta T = T_m - T_m^0$, where T_m is the transition temperature of DMPC MLV prepared in the presence of compound and T_m^0 is the transition temperature of pure DMPC MLV. r values are the transition temperature of DMPC MLV prepared in the presence of compounds at 0.12 molar fraction and represent the maximum interaction between compounds and MLV.

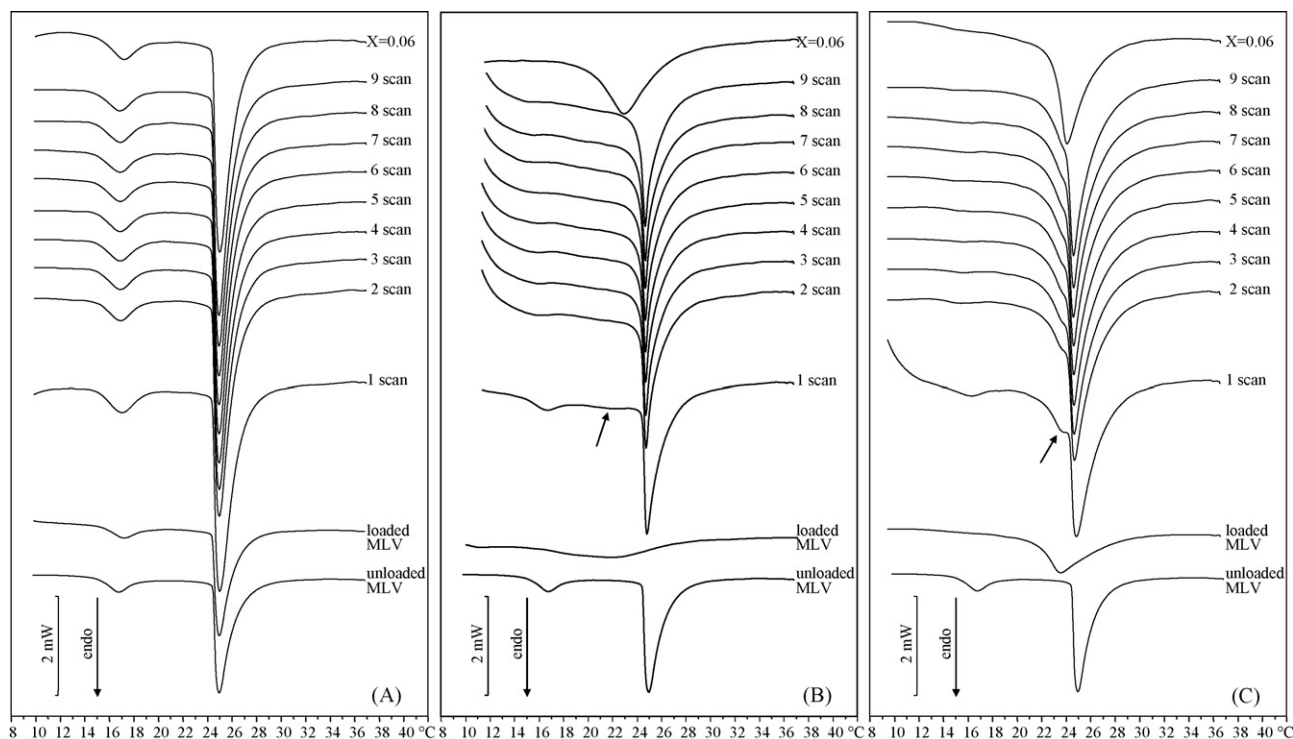


Fig. 8. Calorimetric curves, in heating mode, of DMPC MLV (unloaded MLV) left in contact with DMPC MLV prepared in the presence of (A) acyclovir, (B) squaleneCOOH, (C) squalenoyl-acyclovir at 0.12 molar fraction (loaded MLV), at increasing incubation periods.

ture but the effect exerted by SQACV is a little bigger with respect to squaleneCOOH. It indicates that SQACV is absorbed more than squaleneCOOH by the biomembrane models. SqualeneCOOH does not dissolve in the aqueous medium and, consequently, cannot be absorbed by the MLV. SQACV, being more hydrophilic than squaleneCOOH, dissolves, even if in a small amount, in the aqueous medium, reaches the MLV surface and goes inside the phospholipid bilayers.

3.3. Lipophilic carrier/biomembrane model transfer experiments

The second kind of experiments was aimed at evaluating the capability of the compounds to be transferred from a lipophilic carrier to the biomembrane models. For this purpose, each compound (0.12 molar fraction) loaded MLV (which can mimic a lipophilic carrier) were left to incubate for increasing periods of 1 h, at 37 °C, with unloaded MLV (which mimic the receptor biomembrane models). The compound transfer was evaluated by the calorimetric scans carried out during the first heating and after each incubation period of 1 h (including also the periods of heating and cooling above the transition temperature). If the transfer of the compounds from loaded liposomes to unloaded liposomes happened, a flux of compound should take place that will stop only when the compound concentration equilibrium between the loaded MLV and the initially unloaded MLV is reached. At this stage the T_m value will be the same as that of MLV loaded with a 0.06 molar fraction of compound prepared as described in “Compounds/MLV interaction”. The calorimetric curves (Fig. 8A–C) are compared with that of the sample that were left in incubation (loaded and unloaded MLV) and with that of the curve of the MLV prepared in the presence of compound at 0.06 molar fraction. In the case of acyclovir (Fig. 8A) no modification, as expected, are visible in the calorimetric curves, in fact acyclovir, as seen in the experiments on the interaction compounds/MLV, does not interact with the phospholipid bilayers. Concerning squaleneCOOH (Fig. 8B), the first calorimet-

ric curve (1st scan), obtained from the heating of the sample to the first isothermal period, shows: a pretransition peak (at about 17 °C), a broad small peak (from about 20 to 24 °C) belonging to the loaded MLV, and a main peak (at about 25 °C) belonging to the unloaded MLV. In the curves of the successive scans (at increasing incubation times), the pretransition peak and the broad small peak gradually decrease, the main peak undergoes a very small shift towards lower temperature indicating that squaleneCOOH transfers from the lipophilic carrier to the biomembrane models. The transfer is very slow and incomplete; in fact the molecule, due to its lipophilic nature and, then, to its high affinity for the phospholipid molecules, remains in the MLV bilayers. With regard to SQACV (Fig. 8C), in the first calorimetric curve (1st scan) three regions are present: a pretransition peak (at about 17 °C), a well defined shoulder (at about 23.5 °C) due to the loaded MLV, and a main peak (at about 25 °C) due to the unloaded MLV; in the successive scans, as the incubation time passes, the pretransition peak and the shoulder decrease almost disappearing whereas the main peak shifts towards lower temperature meaning that SQACV is able to transfer, even if not completely, from the carrier to the biomembrane models.

Summarizing the results, it can be concluded that the conjugation of squalene to acyclovir with the formation of a lipophilic prodrug permits a strong interaction with the biomembrane models; the prodrug is scarcely taken up by the biomembrane models but when loaded in a lipophilic carrier the prodrug is gradually transferred to the biomembrane models. These results could help to achieve the desired physicochemical characteristics for the preparation of effective types of liposomal carriers.

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