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Phospholipid-detergent systems: effects of polysorbates on the release of liposomal caffeine☆

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Abstract

It is well known that surfactants are capable of interacting with phospholipid vesicles leading to different aggregated structures and finally to mixed micellar systems. By means of diffusion experiments with Valia-Chien cells the effect of different vesicular structures (MLV and SUV) and that of three polysorbates on the release of a model drug (caffeine) from various vesicle formulations was studied. Obtained results indicate that a remarkable delayed/sustained caffeine release is obtained only with the MLV preparations and that the presence of increasing surfactant concentrations initially leads to a further decrease of drug delivery rate and then to a faster release that reaches a maximum when only mixed micelles are present. The variation of the observed effects with the different tested surfactants, in relation to their lipophilicity (HLB values), is also discussed.

Keywords: Liposomes; MLV; SUV; Mixed micelles; Diffusion; Sustained release

1. Introduction

Amphiphiles in aqueous solutions can form different aggregated structures such as multilamellar vesicles (MLV), small and large unilamellar vesicles (SUV, LUV) and various types of micelles. Furthermore, interactions between identical or different aggregates may result in membrane fusion and mutual vesicle–micelle conversions. In particular, mixed systems of double-chain and single-chain amphiphiles are well known to undergo a transformation from vesicles to micelles as the single-chain mole fraction is gradually raised, and the coexistence of vesicles and micelles has also been reported [1–3]. The interaction of a surfactant with phospholipid liposomes takes place through three main steps [4], as surfactant concentration increases. In a first stage surfactant monomers are incorporated within the lipid bilayer, with a corresponding increase of vesicle dimensions, then phospholipids are gradually solubilized leading to the coexistence of liposomes and mixed micelles, finally a complete solubilization occurs and only mixed micelles are present. These different stages can be easily followed by turbidity and/or light scattering measurements and two detergent concentrations, corresponding to the maximum (i.e. detergent-saturated vesicles, C^{sat}) and to the minimum (i.e. completely disrupted liposomal structures, C^{sol}) turbidity values, can be identified. A typical trend of the turbidity of a SUV dispersion as a function of surfactant concentration is reported in Fig. 1.

Although, as reported above, phospholipid-detergent systems have been widely investigated for several years, at present it still remains unclear as to how the release of loaded drugs can be affected by their inclusion in surfactant-modified vesicles (SUV or MLV) and/or by their partition between vesicles and micelles coexisting in the same formulation. The aim of this work is to investigate the in vitro release of a model drug (caffeine) from different liposomal preparations (SUV, MLV) and the effects of some of the most frequently employed surfactants (i.e. polysorbates) on the release of liposomal caffeine.

☆ Dedicated to Professor Antonio Maccioni.

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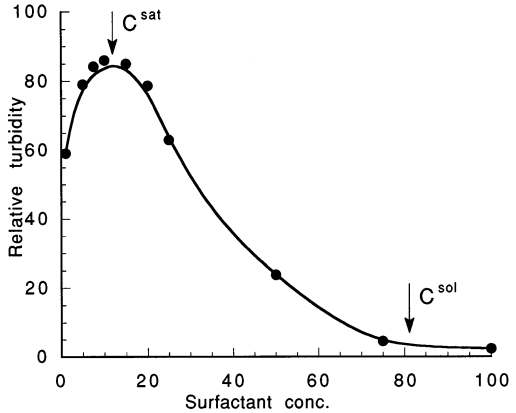

Fig. 1. Effect of increasing surfactant concentration on the turbidity of a liposome dispersion. Approximate points corresponding to C^{sol} and C^{sat} values are indicated by an arrow.

2. Materials and methods

A total of 90% pure enriched soya phosphatidylcholine (Phospholipon 90-Nattermann Phospholipids GmbH-P90) was used for vesicle preparation and 99% pure L-α-phosphatidylcholine from egg yolk (type III E-Sigma-EPC) was employed when the phospholipid was needed as reference, thus with a higher degree of purity. Analytical grade caffeine, polyoxyethylene (20) sorbitan monolaurate (polysorbate 20-TW20), polyoxyethylene (20) sorbitan monostearate (polysorbate 60-TW60) and polyoxyethylene (20) sorbitan monooleate (polysorbate 80-TW80) were purchased from Aldrich. Solvents and all other products employed for the present investigation were of analytical grade. When needed, special HPLC grade reagents (Carlo Erba) were used and distilled water was further purified with a Milli-Q system (Millipore).

Sonication was performed with a Soniprep 150 apparatus (MSE, Crowley) equipped with an exponential microprobe, operating at 23 kHz and at an amplitude of 6 μm.

Phospholipids B test kit (Wako Chemicals GmbH) was used for quantitative determinations of these substances.

Vesicles containing the model drug were prepared according to the following techniques.

2.1. Unilamellar liposomes (SUV)

The appropriate amounts of P90 (25 or 50 mg/ml) were completely dissolved in about 10 ml of methanol. The solvent was vacuum evaporated to leave a thin film inside the vessel. Five milliliters of caffeine–water solution ($1–7.5 \times 10^{-4}$ M) were added, the mixture was gently shaken for 1 h and sonicated, under a nitrogen stream, for 30 min (six times for 5 min). The temperature was maintained at 15–20°C by means of a water bath. SUV were finally purified by filtration (Supor-450, pore size 0.45 μm, Gelman Science). Caffeine and phospholipid concentrations, determined before and after filtration, indicated that over 96% of each substance was always recovered in the liposomal preparation.

2.2. Multilamellar aggregates (MLV)

Prepared according to the same procedure described above with the only difference that the mixture was not sonicated but vortexed for 5 min before use, and no final filtration was carried out.

2.3. Mixed systems

Prepared following the same methods described above and using different polysorbate/P90 molar ratios ranging from 0.03 to 2.33 (i.e. from below C^{sat} to above C^{sol}) calculated according to an average molecular weight of 800 for the phospholipid.

The same procedures were used for SUV and MLV preparations with pure L-α-phosphatidylcholine. Furthermore, for an appropriate comparison, caffeine–polysorbate micellar dispersions and free caffeine water solutions were also prepared.

Vesicle dimensions were determined, at 25°C, by dynamic light scattering, operating at a scattering angle of 90° and 150 mV (ALV-5000, ALV GmbH), while reproducibility among the different preparations was checked by turbidity determinations using a Hitachi U 2000 spectrophotometer working at 600 nm.

2.4. In vitro release studies

Caffeine release from the various formulations was evaluated using Valia-Chien cells. The donor and the receptor compartments were separated by a cellulose membrane (Visking tubing, cut-off 12 000) and had a volume of 3.0 ml. The receptor compartment was filled with distilled water and the donor compartment with a vesicular dispersion and/or micellar solution of caffeine. A caffeine water solution was used as reference. The experiments were performed under magnetic stirring at 50 rpm and at 25°C. Samples of the receiving solution were withdrawn at fixed time intervals and replaced with an equal volume of distilled water.

Quantitative determinations of caffeine were carried out with a HP 1050 series liquid chromatograph (Hewlett–Packard), using a RP 18 column (25.0 cm × 4.6 mm i.d.); the mobile phase was a methanol–water mixture (55:45 v/v); the flow rate was 1.0 ml/min and the temperature was maintained at 25°C. Theophylline was used as the internal standard and caffeine was determined by means of an UV detector set at 273 nm.

All reported data represent the mean values of at least three separate experiments that always presented a

good reproducibility (i.e. $\pm 4\%$) and refer to the experiments carried out with an initial caffeine concentration in the donor compartment of 1×10^{-4} M. Higher caffeine concentrations always showed the same type of results that is given here and the same effect of the presence of surfactant; nevertheless, as expected because of the higher percentages of non entrapped drug, the differences that were observed in the various formulations were less evident.

3. Results and discussion

Caffeine was chosen as a model drug because it is sparingly soluble in water (2.13 g/100 ml at 25°C) and in organic solvents as indicated also by its low octanol/water partition coefficient ($\log P_{ow} = -0.07$), thus it should be capable to enter into a liposomal structure both in the lipophilic bilayer and in the hydrophilic core, but should not be irreversibly captured—thus not released at all—by the phospholipid vesicles. At the same time its solubility is sufficient to prepare water solutions to be used as reference. Furthermore, xantines, and caffeine in particular, were shown to be active in hyperproliferative skin diseases, especially when administered topically using liposomal carriers [5]. Liposomal caffeine is also present in several cosmetic formulations.

It has been stated [6] that sonication of phospholipid dispersions leads mainly to small unilamellar vesicles; in our studies caffeine-loaded SUV liposomes had an average size of 75 ± 8 nm when the P90 concentration was 25 mg/ml and were slightly bigger (89 ± 8 nm) for the higher phospholipid concentration (50 mg/ml). MLV were from five to ten times bigger than SUV, often with an irregular size distribution. Empty vesicles, prepared in the same experimental conditions, were always slightly smaller than those loaded with the model drug. No appreciable differences in mean diameter values were observed when the vesicles were prepared with pure L-α-phosphatidylcholine.

As it is possible to observe from Fig. 2, MLV structures always delayed caffeine release from the diffusing compartment, especially when vesicles were prepared using the highest P90 concentration (50 mg/ml). According to this result, the reduction of delivery rate is to be related to the amount of non-available drug actually included within the vesicular structures; thus, as indicated in the figure, when the phospholipid concentration increased a corresponding decrease of caffeine release rate was obtained. On the contrary, SUV formulations generally showed release profiles quite similar to those observed with the reference free caffeine solution.

According to the physico–chemical properties of the entrapped drug and to the actual structure of the

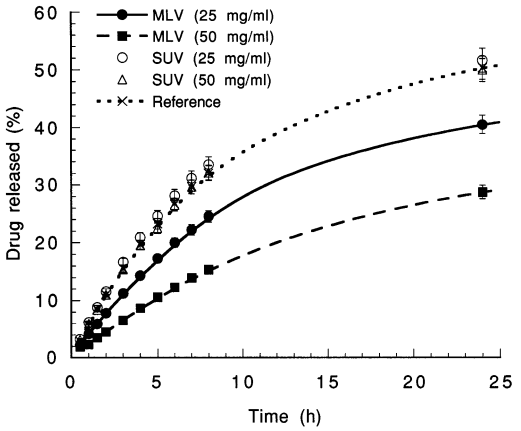

Fig. 2. Cumulative release (%) of caffeine from the donor compartment containing different liposomal formulations. Release from a water solution of caffeine is also reported as reference. For a better visualization of the plot, no curve fit of SUV formulations is reported. The initial caffeine concentration in the donor compartment was 1×10^{-4} M.

vesicles, unexpected and apparently conflicting results have often been reported on in vitro and/or in vivo activity of different liposomal formulations [7,8]; thus the remarkable difference of behaviour observed with the MLV and SUV preparations is not to be considered as an anomalous result, and several explanations have been proposed for a better understanding of such release profile variations between multilamellar and unilamellar liposomes [8,9].

The trend of caffeine release was also differently affected by the presence of the surfactant when vortexed or sonicated mixed systems were used. In fact, as the release from SUV formulation is not appreciably different from that observed from free caffeine solutions, no significant variations were obtained when polysorbates were present in the diffusing solution. On the contrary, the addition of polysorbates in the MLV

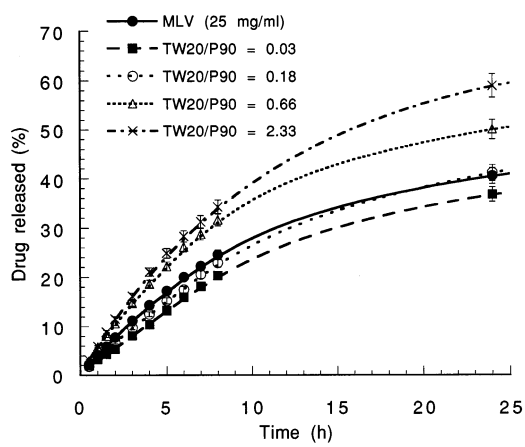

Fig. 3. Effect of increasing polysorbate 20 concentrations, reported as TW20/phospholipid molar ratio, on the cumulative release (%) of caffeine from a MLV (25 mg/ml) formulation. The initial caffeine concentration in the donor compartment was 1×10^{-4} M.

formulations led to remarkable modifications of the release profiles of the model drug. As reported in Fig. 3, where caffeine diffusion from MLV mixed systems is reported for various polysorbate 20/phospholipid molar ratios, the presence of the surfactant at low concentrations (i.e. molar ratio TW20/P90 = 0.03) induced a reduction of the diffusion rate while, as surfactant concentration increased, a corresponding faster caffeine permeation was obtained. In fact, as reported above, at low TW20 concentrations (i.e. below C^{sat}) surfactant monomers are incorporated within the lipid bilayers leading to increased vesicle dimensions (Fig. 1) with a corresponding increase of entrapped drug, while the presence of mixed micelles, at higher surfactant concentrations, reduces the amount of entrapped drug and consequently diffusion rate is increased reaching a maximum value when vesicles are completely disrupted and only mixed micelles are present (i.e. above C^{sol}). That this is the case can be further supported by the diffusion results obtained, in the same experimental conditions, with more lipophilic polysorbates (HLB of polysorbate 20, 60 and 80 are: 16.7, 14.9 and 15.0, respectively) that have consequently higher C^{sat} values [2,10]. For an appropriate comparison, an example is reported in Fig. 4 where drug diffusion from MLV/TW80 mixed systems is reported.

Here the presence of the more lipophilic surfactant induced a reduction of the diffusion rate also at a 0.18 TW80/P90 molar ratio; and again at higher surfactant concentrations a corresponding faster caffeine permeation was observed. For an appropriate comparison, in this figure caffeine diffusion from a polysorbate 80 micellar solution of the model drug is also reported. The increased diffusion rates, observed in the presence

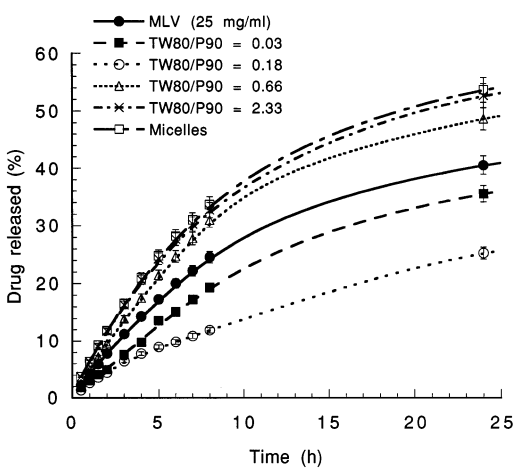

Fig. 4. Effect of increasing polysorbate 80 concentrations, reported as TW80/phospholipid molar ratio, on the cumulative release (%) of caffeine from a MLV(25 mg/ml) formulation. Release from a TW80 micellar solution of caffeine is also reported as reference. The initial caffeine concentration in the donor compartment was 1×10^{-4} M.

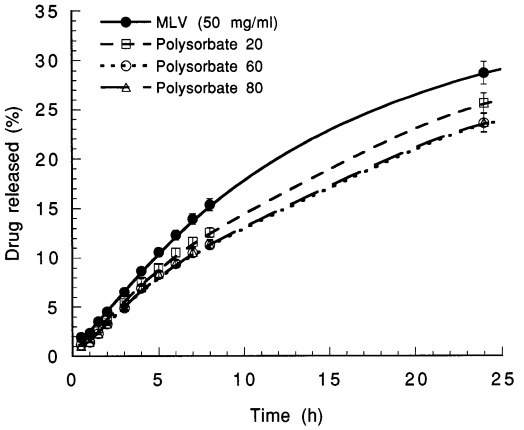

Fig. 5. Effects of the three tested surfactants (polysorbate 20, 60 and 80) at the same molar ratio (i.e. TW/P90 = 0.18) on the release of caffeine from a MLV (25 mg/ml) formulation. The initial caffeine concentration in the donor compartment of 1×10^{-4} M.

of micelles, are to be related to the fast equilibrium between monomers and micellar structures that does not allow the model drug to remain entrapped within the aggregated structures [11].

When the above reported experiments were carried out starting from the MLV preparations obtained with a higher amount of phospholipid (i.e. P90 = 50 mg/ml), the same relative trends of the curves were always observed; but, as expected, slightly higher surfactant concentrations were correspondingly needed to obtain the same type of effects, most probably because the larger MLV can incorporate more surfactant molecules.

In Fig. 5 the release of caffeine from the diffusing compartment, at the same molar ratio of the three tested surfactants (i.e. TW/P90 = 0.18), is reported. The comparison among the obtained curves indicates that drug diffusion rate decreased less with polysorbate 20 (higher HLB value) than with polysorbate 60 and 80 that have similar HLB values (i.e. 14.9 and 15.0) and consequently showed almost overlapping release curves.

Finally, the comparison between the results obtained using vesicles prepared with pure egg phosphatidylcholine and with Phospholipon 90 proved that the origin (egg or soya), and consequently the actual phospholipid composition (i.e. the remarkable difference in fatty acids containing double bonds) [12], never affected significantly the release profile of caffeine.

4. Conclusions

Release kinetics studies indicated that caffeine delivery is remarkably affected by the vesicular structure (MLV showed a different behaviour from that of SUV). The presence of surfactants, such as polysorbates, in the formulations leads first of all to an increase of vesicle dimensions, then to the coexistence of liposomes

and micelles and finally to mixed micelles; these surfactants are consequently capable of inducing noticeable variations of drug release rates according to their concentration with respect to that of the phospholipid; furthermore, such effects can be related also to the degree of lipophilicity (HLB) of the various tested surfactants.

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