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Liposomal drugs dispersed in hydrogels Effect of liposome, drug and gel properties on drug release kinetics

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Abstract

Release of calcein and griseofulvin (GRF) from control (gels in which solutes are dissolved in) and liposomal gels was studied using agarose-assisted immobilization as a technique to separate gels from drug-receptor compartments. Liposomes composed of phosphatidylcholine (PC) or distearoyl-glycero-PC and cholesterol (DSPC/Chol), and incorporating calcein or GRF were prepared by thin film hydration. After cleaning the liposomes they were dispersed in different hydrogels (carbopol 974 [1, 1.5 or 2% (w/w)], hydroxylethyl-cellulose (HEC) [4% (w/w)], or a mixture of the two), and release of calcein or GRF was followed by fluorescence or photometric technique, respectively. Results show that calcein release from liposomal gels is slower compared to control gels, and can be further retarded by using rigid-membrane liposomes (faster release from PC-liposome compared to DSPC/Chol-liposome gels). Additionally, calcein release is not affected by the lipid amount loaded (in the range from 2 to 8 mg/ml), therefore solute loading can be controlled according to needs.

Oppositely, GRF release from liposomal gels is determined by drug loading. At high drug loading levels (compared to GRF aqueous solubility), GRF is released with constant rate from liposomal gels irrespective of liposome type (PC or DSPC/Chol). Thereby, for amphiphilic/lipophilic drugs, drug properties (solubility, log *P*) determine the system behavior.

Calcein and GRF release from control carbopol gels is faster compared to HEC and mixture gels. The same is true for calcein in liposomal gels. Carbopol gel rheological properties were found to be significantly different (compared to the other gels), implying that these characteristics are important for drug diffusion from gels.

Keywords: Liposome; Hydrogel; Release kinetics; Lipid composition; Liposomal gel; Drug; Rheology

1. Introduction

It is well known that liposomes offer many advantages for the delivery and/or targeting of drugs [1,2]. When mucosal or topical delivery of liposome formulations is considered [3,4], it is essential that rheological and/or mucoadhesive properties of the liposome dispersions are adjusted accordingly, depending on the intended route of administration. This can be achieved by adding gelling agents in liposome dispersions [5–7]. Thereby, a drug-in-liposome-in-gel complex formulation is developed. The release of drug molecules from such liposomal gels depends on the stability of the liposomes (membrane integrity and mechanical stability) during their dispersion in the semisolid formulation. This may be determined by the vesicle-membrane rigidity as well as the semisolid system physical properties (as viscosity and rheological properties). The membrane integrity of liposomes is very important when hydrophilic drugs that cannot diffuse across lipid membranes are encapsulated in aqueous compartment of the vesicles. However, when amphiphilic or lipophilic drugs are used, other parameters may also be implicated, as, the lipophilicity of the drug (or its ability to diffuse through lipid membranes), its aqueous solubility that will be the driving force moving drug molecules out of liposomes (for partitioning in the aqueous [dispersion] media).

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In order to evaluate the extent to which such differences can affect the release rate of drugs from drug-in-liposome-in-gel systems we followed the release of two model compounds, one hydrophilic dye (calcein, $M_W = 622.5$) and one lipophilic drug (griseofulvin, $M_W = 352.8$). The effect of liposome-membrane rigidity was evaluated by testing two different lipid compositions: phosphatidylcholine (PC) which is known to form liquid "leaky" membranes, and distearoyl-glycero-PC (DSPC)/cholesterol (Chol) (1:1 mol/mol), a composition that forms very rigid liposomes. Additionally, the effect of gel characteristics was evaluated by using two types of gels: gels composed of carbopol 974, an acrylic acid-based polymer (main component of many commercially available semisolid formulations), as well as a cellulose-based hydroxyethylcellulose (HEC) gel. In has been recently proposed that mixtures of the above two polymer types give gels with improved rheological properties for mucosal (vaginal) administration of drugs [8]. Indeed such mixture gels were found to be more stable towards temperature and pH changes, compared to the two gels when used individually [9]. Thereby, a mixture of the two gels was also evaluated in our studies.

Finally, the rheological properties of the gel formulations evaluated were measured, in order to investigate whether these properties are implicated in the release of drugs from gels.

2. Material and methods

Phosphatidylcholine (PC) and distearoyl-glycero-PC (DSPC) were purchased from Lipoid Gmbh (Ludwigshafen, Germany). The chemical purity of the phospholipids was verified by TLC. Calcein, cholesterol (99%) (Chol), agarose (low melting point) and griseofulvin (GRF) were purchased from Sigma–Aldrich Hellas (Athens, Greece). Hydroxyethylcellulose (HEC) was purchased from Merck (Germany). Carbopol 974 NF (CRB) was kindly provided by Chemix S.A., Athens, Greece.

All solvents used were of analytical or HPLC grade and were purchased from Merck (Germany). All other materials (as salts used for buffer preparation, reagents for lipid concentration determination and surfactants for liposome disruption) were of analytical grade and were purchase from Sigma–Aldrich Hellas (Athens, Greece).

A Shimatzu UV-1205 spectrophotometer was utilized for measurement of GRF and liposomal lipid concentration. All measurements of fluorescence intensity were performed with a Shimatzu RF-1501 spectrofluorimeter at $37 \pm 0.1\,^{\circ}C$ (kept at constant temperature by a Julabo FS18 refrigerating and heating circulator). When appropriate, samples were incubated at $37 \pm 0.5\,^{\circ}C$ in a Julabo SW-20C shaking incubator.

For sample centrifugations a Heraeus Biofuge 28RS (Germany) was used.

2.1. Preparation of liposomes

Multilamellar vesicles (MLV) were prepared by the thin film hydration method [10]. In brief, the appropriate weight of lipid (or lipids) was dissolved in a chloroform/methanol (2:1 (v/v)) mixture, and subsequently evaporated under vacuum until the formation of a thin lipid film. In the case of GRF-containing liposomes the drug was mixed with the lipid solution prior to the formation of the lipid film. The lipid film was hydrated with the appropriate volume of Tris buffer (pH 7.4) or a 100 mM solution of calcein (prepared in the same buffer), at 25 °C in the case of PC liposomes and at 60 °C in the case of DSPC. After complete lipid hydration and formation of liposomes, the vesicle dispersion was placed in a bath sonicator (Branson, USA) for 30 min, for vesicle size reduction. Finally the liposome dispersions were left in peace for annealing structural defects, at a temperature above the lipid transition temperature for 1–2 h.

Separation of liposomes from non-encapsulated molecules was achieved by centrifugation (three spins at 15,000 rpm for 40 min). In the case of GRF-containing liposomes, the liposome dispersion was initially filtered for removal of non-encapsulated and precipitated drug (due to the low aqueous solubility of GRF).

2.2. Characterization of liposome preparations

Lipid concentration of liposomes was measured by the Stewart colorimetric assay [11].

2.2.1. For calcein encapsulating vesicles

Calcein latency was measured [12] in order to be sure that calcein separation is complete and that calcein has not leaked out of vesicles during storage. If % latency was <95, the liposomes were re-centrifuged before being used. Calcein was encapsulated in the vesicles at a quenched concentration (100 mM) and for latency calculation, liposomes (20 μl) were diluted with 4 ml buffer, pH 7.40, and fluorescence intensity (FI) was measured (EM 470 nm, EX 520 nm), before and after vesicle disruption, by addition of Triton X-100 at a final concentration of 1% (v/v). Percent latency (% latency) was calculated from Eq. (1):

$$\%\ \text{latency} = \frac{1.1(F_{AT} - F_{BT})}{1.1F_{AT}} \times 100 \quad (1)$$

where F_{BT} and F_{AT} are calcein fluorescence intensities before and after the addition of Triton X-100, respectively.

2.2.2. For griseofulvin incorporating liposomes

Drug entrapment was routinely estimated by analytical determination of the drug. For this 50 μl of GRF-containing liposomes were solubilized by vortexing in a 50/50 (v/v) mixture of isopropanol with buffer, and the drug concentration was subsequently determined by UV-spectroscopy [13]. Standard solutions of GR were prepared in the appropriate concentration range (in the same media) and spiked with concentrated empty liposomes, in order to have the same final lipid concentration as in samples. Absorption at 280 nm was measured.

2.3. Preparation of gels for liposome dispersion

The appropriate amount of carbopol 974 NF or HEC solid was weighted and added slowly in a citrate buffer solution (pH 5.0), under constant stirring by a paddle stirrer. After addition of