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Evaluation of liposomal formulations containing the antimalarial agent, arteether

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Abstract

Different liposomal formulations containing arteether have been prepared, using the phospholipids, dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC), egg phosphatidylcholine (EPC) or dibenhenoyl phosphatidylcholine (DBPC), alone or in mixtures. The effect of presence of arteether on the liposomal physico-chemical characteristics has been investigated. Arteether was found to change the thermotropic behavior of the liposomal phospholipids that contain a saturated acyl chain such as DMPC and DPPC. On the other hand, arteether did not significantly change the thermotropic behavior of EPC liposomes that contain unsaturated phospholipids. The type of the phospholipid as well as the incorporation of cholesterol in the liposomal bilayer was found to alter the trapping efficiency, liposomal particle size and drug release rate from the liposomes. The trapping of arteether in liposomal vesicles was increased by increasing the acyl chain length of the phospholipid and by addition of cholesterol. EPC liposomes exhibited relatively low trapping efficiency, due to high drug adsorption. Interestingly, liposomal particle size showed a decrease with the increase of acyl chain length in the presence of large molecules of arteether. Incorporation of cholesterol in the liposomal bilayer did not alter the liposomal particle size although it gave lower particle size and distribution. The release of arteether from the liposomal system was characterized by a fast phase for 2 days, followed by a slower phase. The fast phase was the highest with EPC liposomes, indicating the release of the adsorbed drug. Generally, the increase of the acyl chain length as well as the addition of cholesterol caused a decrease in the arteether release rate.

Keywords: Arteether; Liposomal formulation; Phospholipid; Physico-chemical characterization

1. Introduction

Liposomes have many of the requirements for good drug delivery systems as they are relatively non-toxic and bio-degradable (Lopez-Berestein and Fidler, 1989; Vadiei et al., 1989). They have been found to be useful carriers for both hydrophilic and hydrophobic drugs (Gregoriadis

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and Allison, 1980; Lidgate et al., 1988; Akbarieh et al., 1992; Al-Angary et al., 1995). Liposomal encapsulation of a drug can dramatically alter the pharmacokinetic properties of a drug, targeting the drug to particular organs and/or enhance the efficacy of the encapsulated drug (Hwang, 1987; Vadiei et al., 1989; Fielding, 1991). The formulation of an appropriate liposomal system as a carrier for a given drug is dependent on the type of the lipid used and the method of preparation (Taylor et al., 1990; Moghimi et al., 1991; Betageri and Burrell, 1993).

Arteether was recently introduced as a novel semisynthetic antimalarial drug (Brossi et al., 1988) and was found to have a remarkable curative effect against the erythrocytic stage of chloroquine resistant *Plasmodium falciparum* and for cerebral malaria. Arteether is a water-insoluble drug (17 μg/ml at room temperature) and most frequently is given as an oily solution (sesame oil or other vegetable oil) intended for intramuscular injection.

The purpose of this work was to study the physico-chemical characteristics of different liposomal formulations containing arteether and the impact of these characteristics on the stability of the formulations.

2. Experimental

2.1. Materials

Arteether was synthesized from artemisinin (Brossi et al., 1988), the active ingredient isolated from the herb *Artemisia annua* L. The purity of arteether was tested by thin layer chromatogrpahy (TLC) and differential scanning calorimetry (DSC). Dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC), egg phosphatidylcholine (EPC), dibehenoyl phosphatidylcholine (DBPC) and cholesterol (CHOL) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All solvents used for chromatographic analysis of arteether were HPLC grade. All other reagents and solvents were of analytical grade.

2.2. Methods

2.2.1. Preparation of liposomes

Liposomes were prepared with a total lipid concentration of 10 or 20%. The required amount of phospholipids or their mixtures and cholesterol, if needed, were weighed into a stoppered 100-ml pear-shaped glass flask and dissolved in the smallest possible volume of chloroform. The organic solvent was slowly evaporated at reduced pressure, using a rotary evaporator at 40°C, such that a thin film of the dry lipid was deposited on the inner wall of the flask. All remaining traces of chloroform were removed by a jet of dry nitrogen. The dried film was then hydrated in 0.9% sodium chloride (USP) by swirling at a temperature higher by 15°C than the transition temperature of the lipid, in presence of four or five small glass beads, until all the lipid was dispersed (about one hour). The dispersion was then vortexed for one minute to produce multi-lamellar vesicles (MLVs). The preparation of DBC liposomes using the above method was not successful, unless the phospholipid is used in a mixture with other phospholipids and/or CHOL.

2.2.2. Differential scanning calorimetric (DSC) analysis

Transition temperature (T_m) for each liposomal preparation of 20% w/v lipid dispersion was determined using a DSC (Dupont Co., Model 9900, Ct., USA). Pure indium was used to calibrate both temperature scale and quantitative heat changes. The samples were heated from -10 up to 80°C at a scanning rate of 5°C/min. The measured T_m values for vesicles of different compositions are listed in Table 1.

2.2.3. Entrapment of arteether in liposomes

The MLVs were separated from the aqueous solution by centrifugation of the liposomal dispersion at 50 000 $\times$ g (Sorvall Ultracentrifuge, Dupont Co., Model OTD658, CT, USA) for 60 min at 4°C. Free drug in the aqueous solution was determined by an HPLC assay method that was previously developed in our laboratory (Al-Angary et al., 1994). An appropriate volume of the aqueous solution was directly injected in an

HPLC system (Waters Associate, USA). The HPLC separation was achieved with a μ-Bondpack C_{18} column (Waters Association, 10 μm, 30 cm × 3.9 mm), using a mobile phase consisting of a mixture of acetonitrile:water (70:30%, v/v). The eluate was monitored at 216 mm, using a UV detector (Waters Associates, Model 481, USA). The amount of entrapped drug was determined by subtracting the amount of free drug from the total.

Trapping efficiency (TE) was calculated according to the equation:

$$\%TE = \frac{\text{arteether in pellet}}{\text{arteether in liposome suspension}} \times 100$$

2.2.4. Determination of liposomal size

Vesicles sizes were determined using a photomicroscope (Nikon Model UFX-II, Japan) at 1000 × magnification. The proposed method used for the preparation of the liposomes gave large MLVs.

2.2.5. Release of arteether from liposomes

Liposomal preparations of different composition having 10% w/v lipid dispersion were kept at room temperature (20 ± 1°C). The release of arteether from the stored MLVs was monitored for up to 20 days. At a predetermined time intervals and after gentle homogenization, an aliquot of 25 μl from each dispersion was diluted with 10 ml saline and then centrifuged at 50 000 × *g* at 4°C for 60 min. The supernatant was assayed for its content of released arteether, using the HPLC assay method. The produced pellet was dissolved in methanol and examined for the retained drug. Each experiment was repeated four times and the mean values were recorded.

3. Results and discussion

Calorimetric analysis plays a fundamental role in determining both the type and strength of interaction between a lipid and drug molecules. The interaction could be reflected in the change of the thermotropic behavior of the phospholipid with shifting in the transition temperature (T_m). Table 1 shows the effect of presence of arteether on the T_m values for liposomes of different lipid composition. In most cases, the presence of arteether shifted T_m to lower values. Liposomes prepared with the phospholipid DMPC and DPPC in the lipid to drug molar ratio of 1:1 gave T_m equal to 19.41 and 39.48°C compared with 23.56 and 42.38°C for drug-free liposomes, respectively. In addition, arteether caused the characteristic pre-transition of the phospholipid to disappear.

Liposomes formulated with mixtures of phospholipids in molar ratio of 1:1:1 for DMPC, DPPC and arteether showed a decrease in T_m to 30.77°C, compared with 33.18, 29.60 and 35.87°C for drug-free liposomes of the same phospholipid mixture. On the other hand, the presence of arteether in liposomes formulated with EPC, a phospholipid containing an appreciable amount of unsaturated phospholipids, did not show a significant change in T_m values. The T_m value was −2.09°C for drug-free liposomes and −1.96°C

Table 1
Effect of arteether (A) on the transition temperature, particle size, particle size distribution and trapping efficiency of liposomes of different compositions

Liposomes composition (mol ratio)	T_m (°C)	Size ± SD (μm)	%TE
DMPC	23.56	—	—
DPPC	42.38	—	—
DMPC:A (1:1)	19.41	4.76 ± 2.06	48.45
DPPC:A (1:1)	39.48	4.38 ± 1.72	56.22
DMPC:DPPC:A (1:1:1)	30.77	3.71 ± 1.41	59.05
DMPC:DPPC:Chol:A (1:1:1:1)	—	3.79 ± 1.13	63.80
EPC	−2.06	—	—
EPC:A	−1.98	3.17 ± 1.69	46.17
EPC:Chol:A (1:0.5:1)	—	3.33 ± 1.33	48.89
EPC:Chol:A (1:1:1)	—	3.35 ± 1.23	51.64
DPPC:DBPC:Chol:A (1:1:1:1)	—	3.28 ± 0.92	61.82
DPPC:DBPC:Chol:A (1:1:2:1)	—	0.21 ± 0.76	67.56
DBPC:Chol:A (1:1:1)	—	3.25 ± 1.18	79.21
DBPC:Chol:A (1:1:2)	—	3.20 ± 1.03	82.33

in presence of arteether at a 1:1 molar ratio of EPC to arteether.

The above results would indicate that arteether alters the thermogram obtained from the gel-liquid crystal transition formed by phospholipids containing saturated acyl chain such as DMPC and DPPC, or their mixtures. Generally, the change in T_m was small in the presence of arteether and this could be explained by the ability of arteether molecules to be adsorbed at the lipid/water interface beside the deep partitioning in the hydrophobic bilayer. This is based on the findings obtained from the study of micellar solublization of arteether, where it was suggested that the site of solubilization for arteether not only involves the incorporation into the micellar interior, but also may be substantially due to adsorption at the micellar/water interface (Krishna and Flanagan, 1989). Solubilization behavior also suggested the possible interaction between the peroxide group in arteether molecules and the polar head group of ionic micelles. This may explain the nonsignificant effect of arteether on the thermotropic characteristics of EPC liposomes that contain unsaturated phospholipids where massive adsorption of arteether is possible.

The trapping efficiency (TE) of different liposomal systems to arteether is summarized in Table 1. In the preparation of the liposomal formulations, co-evaporation of the lipid and lipophobic compound from organic solvents has been used to obtain the highest incorporation (Juliano and Daoud, 1990). It was clear that TE is strongly dependent on the lipid composition of the liposomes. The TE value was 48.45% for DMPC liposomes and 56.22% for liposomes prepared with DPPC that contain a longer acyl chain. Equimolar ratios of DMPC and DPPC was found to give high TE values and this was in agreement with previous studies on other lipophilic drugs (Wu et al., 1978; Akbarieh et al., 1992) where, it was found that drug encapsulation reaches the highest level when phospholipids are present at similar ratios.

In using the phospholipid EPC, of higher molecular weight and having unsaturated phospholipids, the trapping efficiency was only 46.17 and this may be due to the higher ability for the arteether molecules to be loosely adsorbed at the water/phospholipid bilayer interface. The addition of CHOL, or the increase of its ratio in the liposomal bilayer, also altered the TE values (Table 1) where the ability of the bilayer for the uptake of hydrophobic drug molecules is dependent on the dimension and molecular structure of the bilayer (Szoka and Papahadjopoulos, 1980).

Liposomal preparations containing the phospholipid DBPC in a mixture with other phospholipids, or in presence of CHOL, gave the highest TE compared with those of DMPC, DPPC and EPC (Table 1). This is in agreement with the idea of increase of the TE values with the increase of the acyl chain length.

Table 1 also shows the mean liposomal particle sizes for different liposomal composition. In this study, the method used for the preparation of the liposomes gave large MLVs with mean diameter and particle size distribution depending on the type of lipid used. Generally, no cluster or crystals were observed for all the investigated preparations. Interestingly, it was found that as the acyl chain of the lipid increases the liposomal size decreases in the presence of the relatively large molecules of arteether. The mean particle sizes were 4.76 $\pm$ 2.06, 4.38 $\pm$ 1.72 and 3.17 $\pm$ 1.29 μm for DMPC, DPPC and EPC, respectively. It was also clear that the presence of CHOL did not significantly change the particle size of the liposomes, but it gave a lower particle size distribution indicating more ordered structure. Mixtures of phospholipids gave smaller particles and DBPC gave relatively the smallest particle sizes in presence of other phospholipids.

The release of arteether from the liposomal vesicles was found to be dependent on both the liposomal composition and the amount of added CHOL. The release of arteether was characterized by two phases, an initially fast phase (within 2 days) followed by slower release phase. The initial release of arteether from EPC liposomes was the highest compared with other phospholipids followed by much slower release. This could indicate that the fast release phase is due to the release of drug loosely adsorbed at the water/lipid interface.

The release rates of arteether from different types of liposomes after 2 days for up to 20 days

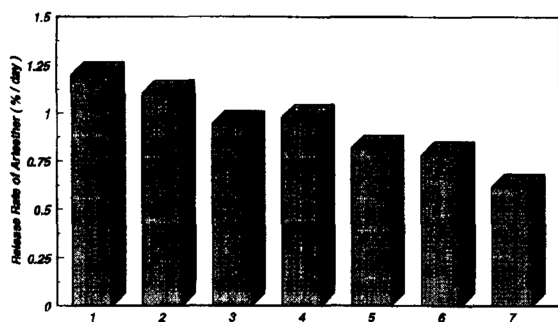

Fig. 1. Release rate of arteether from liposomes of different compositions. Liposomes composition (mol ratio): (1) DMPC; (2) DPPC; (3) EPC; (4) DMPC:DPPC (1:2); (5) DBPC:DPPC (1:1); (6) DBPC:CHOL (1:1); (7) DBPC:CHOL (1:2).

at 20 ± 1°C are shown in Fig. 1. It was clear that the increase in the length of the lipid acyl chain length was accompanied by a decrease in the release rate of arteether from the liposomes. The release rates were 1.20, 1.013 and 0.944%/day for DMPC, DPPC and EPC liposomes, respectively. In the mean time, the use of a mixture of the phospholipids, DMPC and DPPC, in equal ratio gave a release rate equal to 0.979%/day indicating more stable liposomes. The release rate of arteether from liposomes prepared with mixtures of DBPC and DPPC with a 1:1 molar ratio was 0.818%/day, while it was only 0.783%/day when cholesterol was added to DBPC in ratio of 1:1 (low CHOL) and 0.616% when CHOL was used in a ratio of 1:2 (high CHOL). These results showed that the increase of CHOL in the bilayer composition would lead to a decrease in the leakage rate of arteether, where CHOL molecules conferred structural stability and gave a more ordered structure bilayer.

In conclusion, the hydrophobic antimalarial agent arteether was successfully incorporated in some liposomal formulations. In vitro studies demonstrated that drug trapping as well as liposomal stability could be enhanced using mixtures of saturated phospholipids and/or incorporating cholesterol in liposomal bilayer. Further in vivo studies on the prepared arteether liposomes are in progress in our laboratory.

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