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Research paper

A study of the formulation design of acoustically active lipospheres as carriers for drug delivery

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Abstract

Acoustically active lipospheres (AALs) were prepared using perfluorocarbons and coconut oil as the cores of inner phase. These AALs were stabilized using coconut oil and phospholipid coatings. A lipophilic antioxidant, resveratrol, was the model drug loaded into the AALs. AALs with various percentages of perfluorocarbons and oil were prepared to examine their physicochemical and drug release properties. Co-emulsifiers such as Brij 98 and Pluronic F68 (PF68) were also incorporated into AALs for evaluation. AALs with high resveratrol encapsulation rates (~90%) were prepared, with a mean droplet size of 250–350 nm. The AALs produced with perfluorohexane as the core material had larger particle sizes than those with perfluoropentane. Resveratrol in these systems exhibited retarded drug release in both the presence and absence of plasma in vitro; the formulations with high oil and perfluorocarbon percentages showed the lowest drug release rates. The addition of PF68 slightly but significantly reduced resveratrol delivery from the AALs. Ultrasound treatment of 1 MHz produced an increase in the drug release from the systems, illustrating the drug-targeting effect of the combination of AALs and ultrasound.
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Keywords: Acoustically active lipospheres; Resveratrol; Perfluorocarbons; Drug delivery; Formulation design

1. Introduction

An important prerequisite for the success of applying pharmacologically active drugs is site specificity. Drug encapsulation systems such as liposomes and emulsions have been introduced as parenteral drug carriers offering sustained release and organ targeting [1,2]. Microbubbles represent a new class of parenteral formulations with both diagnostic and therapeutic applications. Microbubbles are comprised of spherical voids or cavities filled by a gas. For medical applications, microbubbles are generally stabilized by a coating material such as phospholipids, surfactants, albumin, or polymers [3]. One prototypical kind of microbubbles using an oily layer around the microbubbles is referred to as acoustically active lipospheres or AALs [4]. Microbubbles can be used to deliver a drug or gene to a specific area of interest, and then ultrasound is used to burst the microbubbles, producing site-specific delivery [5].

Although many studies have investigated the in vivo or clinical advantages of microbubbles or AALs, no investigation of the formulation design has been conducted. The aim of the present study was to explore the effects of perfluorocarbon and oil on the physicochemical characteristics, drug delivery, and safety of AALs. The effect of incorporation of a co-emulsifier was also examined. The model drug used in this study was resveratrol. Resveratrol, a natural product from red wine, can play an important role in the therapy for cardiovascular diseases and cancers [6]. The oral bioavailability and initial half-life (8–14 min) are poor, leading to an irrelevant in vivo effect by oral administration compared to its powerful in vitro efficacy [7]. Hence other routes

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such as a parenteral injection should be considered in order to obtain better therapeutic benefits. Several phase I clinical trials are currently underway in several locations [8]. Resveratrol, like paclitaxel, is a typical drug with low aqueous solubility. More-extensive clinical use may be somewhat delayed due to a lack of appropriate delivery vehicles.

Lipid-coated microbubbles are potentially interesting delivery systems because of their ability to incorporate drugs for delivery aim [9]. Cavitation of microbubbles with ultrasound can be used to treat vascular thromboses and deliver drugs [3]. Hence it may be feasible to encapsulate resveratrol in AALs so that its pharmacological activity can be delivered to the cardiovascular system. Understanding how the formulation variables influence drug release and targeting may help predict the in vivo behavior of targeted AALs and the design of successful preparations.

2. Materials and methods

2.1. Materials

Perfluorohexane, coconut oil, and Pluronic F68 (PF68) were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). Perfluoropentane (Fluorinert® Fluid PF-5050) was obtained from Fluka Chemie (Buchs, Germany). Hydrogenated soybean phosphatidylcholine (Phospholipon® 80H) was supplied by American Lecithin Company (Oxford, CT, USA). Brij 98 (polyoxyethylene glycol mono-*n*-dodecyl ether) was from Acros Organics (Geel, Belgium). The cellulose membranes (Cellu-Sep® T3, with a molecular weight cutoff of 3500) were supplied by Membrane Filtration Products (Seguin, TX, USA).

2.2. Preparation of AALs

Soybean phosphatidylcholine (2.8%, w/v in the final product), cholesterol (1.2% in the final product), co-emulsifier (0.8% in the final product), and resveratrol (0.2% in the final product) were dissolved in an appropriate volume of chloroform–methanol (2:1). The organic solvent was evaporated in a rotary evaporator at 50 °C to obtain a thin film, and solvent traces were removed by maintaining the lipid film under a vacuum for 6 h. The film was hydrated with double-distilled water using a probe-type sonicator (Sonics and Materials VCX 600, CT, USA) by a 35-W intensity for 10 min at 60 °C. Then coconut oil (1.8% or 18% in the final product) and perfluorocarbon (16% or 32% in the final product) were added to the system, followed by high-shear homogenization (Pro Scientific Pro250, Monroe, CT, USA) for 5 min and sonication by a probe-type sonicator for 10 min at room temperature.

2.3. Droplet size and zeta potential

The mean particle size (*z*-average) and zeta potential of the AALs were measured by a laser scattering method (Malvern Nano ZS® 90, Worcestershire, UK). The formulations were diluted 100-fold with double-distilled water before the measurement. The determination was repeated three times/sample for three samples.

2.4. Resveratrol encapsulation in AALs

The AALs were centrifuged at 48,000*g* and 4 °C for 30 min in a Beckman Optima MAX® ultracentrifuge (Beckman Coulter, USA) in order to separate the incorporated drug from the free form. The supernatants were analyzed by HPLC for the free form to determine the encapsulation percentage.

2.5. HPLC analysis of resveratrol

The HPLC system for resveratrol included a Hitachi L-7110 pump, a Hitachi L-7200 sample processor, and a Hitachi L-7400 UV/visible detector. A 25-cm-long, 4-mm inner diameter stainless RP-18 column (Merck, Darmstadt, Germany) was used. The mobile phase was a methanol: pH 2.6 aqueous solution adjusted by acetic acid (45:55) at a flow rate of 1.0 ml/min. The UV/visible detector was set at 310 nm.

2.6. Evaporation of AALs

Two milliliters of AALs was pipetted into a cylindrical vial with an opening diameter of 2.5 cm. The sample vial was positioned in an incubator at 37 °C. At determined periods, the vial was weighed and the AAL weight remaining in the vial (%) was calculated. The total duration of the experiment was 12 h. The water and neat perfluorocarbons were also examined as the controls.

2.7. Erythrocyte hemolysis

Blood samples were obtained from a healthy donor by venipuncture and collected into test tubes containing 124 mM sodium citrate (one volume of sodium citrate solution + nine volumes of blood). The erythrocytes were immediately separated by centrifugation at 2000*g* for 5 min and washed three times with four volumes of a normal saline solution. Erythrocytes collected from 1 ml of blood were resuspended in 10 ml of normal saline. Immediately thereafter, 2.5 ml of 2% (w/v) AALs in normal saline was incubated with 0.1 ml of the erythrocyte suspension. Incubations were carried out at 37 °C with gentle tumbling of the test tubes. After 1 h of incubation, the samples were centrifuged for 5 min at 2000*g*. The absorbance of the supernatant was measured at 415 nm to determine the percentage of cells undergoing hemolysis. Hemolysis induced with double-distilled water was taken as 100%.

2.8. In vitro drug release

Resveratrol release from the AALs was measured using a Franz diffusion cell. The cellulose membrane was mounted between the donor and receptor compartments. The

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