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Successful co-encapsulation of benzoyl peroxide and chloramphenicol in liposomes by a novel manufacturing method - dual asymmetric centrifugation

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ABSTRACT

Encapsulation of more than one active pharmaceutical ingredient into nanocarriers such as liposomes is an attractive approach to achieve a synergic drug effect and less complicated dosing schedules in multi-drug treatment regimes. Liposomal drug delivery in acne treatment may improve drug efficiency by targeted delivery to pilosebaceous units, reduce adverse effects and improve patient compliance. We therefore aimed to co-encapsulate benzoyl peroxide (BPO) and chloramphenicol (CAM) into liposomes using the novel liposome processing method – dual asymmetric centrifugation (DAC). Liposomes were formed from soybean lecithin, propylene glycol and distilled water (2:1:2 w/v/v ratio), forming a viscous liposome dispersion. Liposomes containing both drugs (BPO-CAM-Lip), single drug (BPO-Lip and CAM-Lip), and empty liposomes were prepared. Drug entrapment of BPO and CAM was determined by a newly developed HPLC method for simultaneous detection and quantification of both drugs. Encapsulation of around 50% for BPO and 60% for CAM respectively was obtained in both single-drug encapsulated formulations (BPO-Lip and CAM-Lip) and co-encapsulated formulations (BPO-CAM-Lip). Liposome sizes were comparable for all liposome formulations, ranging from 130 to 150 nm mean diameter, with a polydispersity index <0.2 for all formulations. CAM exhibited a sustained release from all liposomal formulations, whereas BPO appeared retained within the liposomes. BPO retention could be attributed to its poor solubility. However, HaCaT cell toxicity was found dependent on BPO released from the liposomes. In the higher concentration range (4% v/v), liposomal formulations were less cytotoxic than the corresponding drug solutions used as reference. We have demonstrated that DAC is a fast, easy, suitable method for encapsulation of more than one drug within the same liposomes.

1. Introduction

Acne vulgaris (or simply acne), is a chronical inflammatory skin disease, affecting approximately 90% of the global population during adolescence. However, the skin condition might also continue into adulthood, having a severe impact on self-esteem and general quality of life (Williams et al., 2012). Current guidelines for management of acne recommend combination therapy with topical retinoid and benzoyl peroxide (BPO) as first-line treatment in mild to moderate acne (Walsh et al., 2016; Zaenglein et al., 2016).

The use of multiple drugs in combination to achieve a synergistic effect in treatment of diseases is a well-known strategy for improved therapeutic outcome, and has been adapted as standard first-line treatment in several diseases (Durante-Mangoni et al., 2014; Fischbach, 2011; Landewé et al., 2002). The use of combination therapy is also likely to increase in the future as the emergence of antibiotic resistant pathogens is associated with therapeutic failure, forcing us to develop new drugs and treatment strategies in order to assure future availability of treatment for these pathogens (Durante-Mangoni et al., 2014; Walsh et al., 2016). The drawback of conventional combination therapy is that it is more complex compared to monotherapy and often involves the use of several dosage forms that increase the possibility of patient non-adherence during the treatment. Co-encapsulation and co-delivery of drugs using nanocarriers such as liposomes has the potential to simplify the therapy, since drugs incorporated into the same carrier will be delivered to the target simultaneously.

Today, chloramphenicol (CAM) is mainly used for treatment of eye and ear infections, as its oral and intravenous use are limited by its bone marrow toxicity. However, topical administration of CAM for treatment of local invasive skin infections offers rapid delivery, and since nanocarriers might circumvent the limitations of the drug associated with systemic toxicity, CAM is already recognized as a good candidate for encapsulation into nanocarriers for topical treatment of skin infections (Ingebrigtsen et al., 2016; Kalita et al., 2015). Moreover, it has been demonstrated that old

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antibiotics such as CAM remain active against prevalent resistant bacterial isolates owing to their rather low-level use in the past (Fayyaz et al., 2013).

Although BPO is preferred first-line therapy for the treatment of mild to moderate acne, it is known that BPO might cause local adverse reactions such as cutaneous irritation or dryness, erythema and scaling (Foti et al., 2015). In addition, BPO is also prone to degradation and is unstable in solution (Chellquist and Gorman, 1992).

Liposomes were first suggested as a topical drug delivery system by Mezei and Gulasekharam (1980), and have been studied quite intensively as skin nanocarriers since then; however co-encapsulation of multiple drugs into liposomes is a relatively new approach. Recently, several studies have investigated the potential of this approach, and reported liposomal encapsulation of drug combinations such as topotecan/vincristine (Zucker et al., 2012) and irinotecan/doxorubicin for anticancer therapy (Shaikh et al., 2013). The physiochemical properties and solubility limitations of both BPO and CAM in aqueous solutions make them suitable candidates for liposomal entrapment; with log K_{ow}-values of 3.46 and 1.14, respectively. A formulation containing these two drugs co-encapsulated in liposomes would not only be beneficial in terms of overcoming their solubility and stability issues, it would also have the potential synergic therapeutic effect and reduce adverse effects.

Dual asymmetric centrifugation (DAC), is a laboratory mixing system, which differs from conventional centrifugation by adding a secondary rotation around its own axis (Massing et al., 2008). The suitability and versatility of this new technique for liposome production has already been demonstrated in a number of studies (Adrian et al., 2011; Ingebrigtsen et al., 2016; Massing et al., 2008; Meier et al., 2015; Parmentier et al., 2014; Tian et al., 2010). Liposomes can be produced in a gentle way, and aseptic manufacturing is facilitated, as the sample holder is a closed container (Hirsch et al., 2009). Since the technique provides a closed system and eliminates the need of organic solvents, it has several advantages compared to other available production methods (Massing et al., 2008; Wagner and Vorauer-Uhl, 2011). Concentrated vesicular phospholipid gels (VPGs) are formed as an intermediate product in this process, making it especially suitable for obtaining high liposome content in secondary vehicles such as creams, lotions or hydrogels (Ingebrigtsen et al., 2016).

In this study, we aimed to investigate the suitability of DAC for the production of co-encapsulated BPO and CAM in liposomes. In addition, a new HPLC method for simultaneous detection of BPO and CAM was developed to facilitate fast and easy drug quantification during characterization of the investigated formulations. The system's toxicity was compared to single-drug liposomes and corresponding drug solutions.

2. Materials

Acetone, acetonitrile CHROMASOLV®, 99.8% anhydrous acetic acid, ammonium molybdate, Luperox® A75FP (75% benzoyl peroxide; remainder water), chloramphenicol, chloroform, Dulbecco's Modified Eagle's Medium with 1% L-glutamine, ethanol 96%, Fiske-Subbarow reducer agent, isopropanol, monobasic potassium phosphate, RPMI-1640 medium, sodium chloride and Triton X-100 were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Disodium hydrogen phosphate dihydrate, and 30% water-free hydrogen peroxide were purchased from Merck (Darmstadt, Germany). Concentrated sulfuric was the product of May and Baker LTD (Dagenham, England). Propylene glycol was purchased from NMD – Norwegian Medical Depot (Oslo, Norway). Lipoid S 100 (soybean lecithin, >94% phosphatidylcholine) was a kindly provided gift from Lipoid GmbH (Ludwigshafen, Germany).

3. Methods

3.1. Liposome preparation

Liposomes were made by dissolving 200 mg Lipoid S 100 (PC) in 10 ml of acetone:chloroform (1:2 v/v) together with 3 mg BPO (equal to 4 mg Luperox® A75FP) and/or 20 mg of CAM.

For all formulations, the organic solvents were removed at room temperature under a stream of nitrogen using the setup described elsewhere (Ingebrigtsen et al., 2016). The lipid film was hydrated with 100 μl propylene glycol (PG) and 200 μl distilled water. Finally, glass beads (Ø = 2 mm) equal to 50% (w/w) of the total sample weight were added before storage overnight at 4 °C.

Liposome size was reduced by DAC using a Speedmixer (DAC 150.1 FVZ-K Speedmixer, Synergy Devices Ltd., High Wycombe, UK). All samples were processed for 40 min at maximum speed, corresponding to 3500 rpm. The VPGs were diluted with distilled water to produce liposomal dispersions with a total volume of 2 ml, suitable for further characterization.

3.2. Liposome characterization

3.2.1. Entrapment efficiency

Free unentrapped BPO crystals were separated from entrapped liposomal BPO by filtration through a 0.22 μm filter (Acrodisk, Pall Corporation, New York, USA). Total amount of BPO in the filtrate and in the original sample was quantified by HPLC analysis. Drug entrapment of BPO was calculated as described in Eq. (1).

$$\frac{\text{Amount of BPO}_{\text{filtrate}}}{\text{Amount of BPO}_{\text{original sample}}} \times 100 = \text{Entrapment efficiency (\%)} \quad (1)$$

The entrapment of CAM was determined by dialysis: One milliliter of liposomal dispersion was transferred to a dialysis bag (cellulose membrane with a M_w cut-off of 12–14,000 Da, Medicell International Ltd., London, UK). The bag was put into a beaker filled with 500 ml of distilled water (sink conditions). After 4 h of dialysis, CAM concentration in the liposome dispersion was quantified by HPLC. Drug entrapment efficiency of CAM was calculated using Eq. (2).

$$\frac{\text{Amount of CAM}_{\text{dialyzed sample}}}{\text{Amount of CAM}_{\text{original sample}}} \times 100 = \text{Entrapment efficiency (\%)} \quad (2)$$

3.2.2. Size measurements

Size measurements were performed on a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK), using the following settings; sample temperature 25 °C, equilibration time 180 s, and number of runs was set to automatic. All liposome dispersions were diluted 1:100 with filtered distilled water prior to measurements.

3.2.3. Zeta-potential measurements

The zeta-potential of the liposome dispersions was also determined using the Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK), with the same equilibration time and temperature as for the liposome size measurements (3.2.2). All measurements were performed in triplicate, using the automatic setting for drive voltage and number of runs per measurement. All liposomal samples were diluted 1:10 with filtered distilled water prior to measurements.

3.2.4. In vitro drug release

Franz diffusion cells (PermeGear, Bethlehem, USA) with a surface area of 0.64 cm^2 and receptor volume of 5.0 ml were used for the drug release studies. The cells were mounted in a stirrer (V6A-02, PermeGear, Bethlehem, USA) and connected to a heating circulator (F12-ED, Julabo Laboratechnik, Seelback, Germany). All experiments were performed in triplicates at 25 °C using a cellophane membrane (Jøraholmen et al., 2014). Phosphate buffered saline (PBS) (2.98 g/l disodium hydrogen phosphate dihydrate, 0.19 g/l monobasic potassium phosphate, and 8 g/l sodium chloride) with pH 7.4 was the chosen acceptor phase for these experiments. Free drug was removed from the liposome dispersion (as described in Section 3.2.1) prior to testing. The sample volume (both liposome formulations and the control drug