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Nano-transfersomal ciprofloxacin loaded vesicles for non-invasive trans-tympanic ototopical delivery: *In-vitro* optimization, *ex-vivo* permeation studies, and *in-vivo* assessment

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ABSTRACT

Ciprofloxacin is a synthetic fluoroquinolone antibiotic that has been used for systemic treatment of otitis media in adults. It was approved for topical treatment of otorrhea in children with tympanostomy tubes. The aim of this work was to enhance the local non-invasive delivery of ciprofloxacin to the middle ear across an intact tympanic membrane (TM) in an attempt to treat acute otitis media (AOM) ototopically. In order to achieve this goal, ciprofloxacin nano-transfersomal vesicles were prepared by thin film hydration (TFH) technique, using several edge activators (EAs) of varying hydrophilic–lipophilic balance (HLB) values. A full factorial design was employed for the optimization of formulation variables using Design-Expert® software. The optimal formulation was subjected to stability testing, *ex-vivo* permeation studies (through ear skin and TM of rabbits), and *in-vivo* evaluation. Results revealed that the optimal formulation (composed of phospholipid and sodium cholate as an EA at a molar ratio of 5:1) exhibited enhanced *ex-vivo* drug flux through ear skin and TM when compared with the commercial product (Ciprocin® drops). It demonstrated a greater extent of *in-vivo* drug deposition in the TM of albino rabbits relative to Ciprocin®. Consequently, transfersomes could be promising for the non-invasive trans-tympanic delivery of ciprofloxacin.

1. Introduction

Acute otitis media (AOM) is a sudden infection of the middle ear that usually begins as a result of upper respiratory tract pathogens entry to the space of the middle ear *via* the eustachian tube, leading to an inflammation and an effusion (Thomas et al., 2014; Thornton et al., 2011; Zeng et al., 2014). Although it may occur at any age, it is most frequently diagnosed in children (Marchisio et al., 2014; Rosenfeld and Clarity, 1996). In the United States of America, AOM in pediatric patients accounts for more than twenty million visits to physicians, annually (Khoo et al., 2013). Approximately one third of the pediatric populations in the US have suffered at least six episodes of AOM in the first seven years of life, demonstrating the recurrent nature of the disease (Faden et al., 1998). The typical management of AOM usually includes an oral course of a broad spectrum antibiotic (Khoo et al., 2013). A great proportion of antibiotics prescribed in the U.S. (≥25%) are dispensed to treat AOM (Rosenfeld and Clarity, 1996).

To the present day, the use of ototopically delivered antibiotic for the treatment of middle ear infections has been limited to those patients with perforated ear drums or tympanostomy tubes (Koulich et al., 2010). The main reason behind such a limitation is the low permeability of the stratum corneum layer in the tympanic membrane (TM), which presents a potential barrier for trans-tympanic diffusion (Khoo et al., 2013). However, local non-invasive antibiotic delivery across an intact TM to the middle ear may be considered as a promising alternative to systemic antibiotics for the treatment of AOM. In a previous study, an *in-*

Abbreviations: AOM, acute otitis media; TM, tympanic membrane; EA, edge activator; TFH, thin film hydration; PC, L-α-phosphatidylcholine; CH, cholesterol; SA, stearylamine; DP, dicetylphosphate; HLB, hydrophilic–lipophilic balance; EE%, entrapment efficiency; PS, particle size; DI, deformability index; PDI, polydispersity index; ZP, zeta potential; SD, standard deviation; PBS, phosphate buffer saline; TEM, transmission electron microscope; J_{max}, flux of drug at 24 h; ER, enhancement ratio; EAM, external auditory meatus; AUC, area under the curve.

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situ gelling system containing chemical penetration enhancers was developed for trans-tympanic delivery of ciprofloxacin (Khoo et al., 2013). Such a local non-invasive treatment of AOM will present several advantages over the conventional systemic antibiotic therapy such as, enhanced local bioavailability of the drug at the site of action (the middle ear), complete eradication of the pathogenic bacteria and thus, reduced likelihood of recurrent infection, and reduced risk of developing antibiotic resistance that may occur upon oral administration (Khoo et al., 2013).

Ultradeformable liposomes, transfersomes, consist of phospholipids and an edge activator (EA) which increases elasticity of the vesicles (Cevc and Blume, 1992). The ability of these deformable vesicles to cross the skin as a result of their elasticity has been intensively studied (El Maghraby et al., 2000; El Zaafarany et al., 2010). It has been shown that upon non occlusive application to the skin surface, transfersomal vesicles significantly enhance the trans-dermal delivery of drugs that would otherwise penetrate poorly (Cevc and Blume, 1992). Several researches have demonstrated the utility of transfersomes as carriers for various compounds, such as proteins (Paul et al., 1998), insulin (Cevc et al., 1998), corticosteroids (Cevc and Blume, 2004), ketoprofen (Cevc et al., 2008), and anticancer agents (Hiruta et al., 2006). Based on the structural similarities between the skin and the TM, and since these elastic vesicles move towards areas of high water content, it would be expected that a transfersomal formulation could potentially enhance the antibiotic flux across the intact TM into the infected middle ear.

Ciprofloxacin is a synthetic broad-spectrum bactericidal fluoroquinolone antibiotic. It acts by inhibiting the bacterial DNA gyrase, resulting in the degradation of bacterial DNA by exonuclease activity (Campoli-Richards et al., 1988). It is a very widely used antibiotic for the systemic and/or topical treatment of chronic suppurative otitis media in adults (Esposito et al., 1990; Legent et al., 1994). Although contraindicated for systemic use in children because of its effect on bone growth plates, ciprofloxacin was approved for topical treatment of AOM in pediatric patients with tympanostomy tubes (Wall et al., 2009). Because of its well known stability in solutions, its low molecular weight (331.4 Da) and its moderate lipophilicity (log $p = 0.28$) (Moffat et al., 2011), ciprofloxacin was chosen as a good candidate for our study.

Up to date, the use transfersomes to enhance trans-tympanic delivery of bioactive agents has not been investigated yet. In view of the aforementioned, the aim of this work is to enhance non-invasive trans-tympanic delivery of ciprofloxacin into the middle ear *via* ototopical application of nano-vesicular formulation. In order to achieve this goal, ciprofloxacin-loaded transfersomes were prepared by thin film hydration (TFH) technique, using several EAs. The effect of different formulation variables on vesicle characteristics was investigated. In addition, *ex-vivo* permeation studies across intact TM and ear skin of albino rabbits as well as *in-vivo* TM deposition studies were conducted for the optimal transfersomal formulation.

2. Materials and methods

2.1. Materials

Ciprofloxacin was kindly provided by El Nasr Pharmaceutical Company, Cairo, Egypt. L-α-Phosphatidylcholine (PC) from egg yolk, cholesterol (CH), Span 20 (sorbitan monolaurate), Span 60 (sorbitan monostearate), Span 65 (sorbitan tristearate), Pluronic L-121, Pluronic P-123, Pluronic F-127, Pluronic F-68, sodium cholate, sodium deoxycholate, Cremophor EL, and Cremophor RH 40 were purchased from Sigma Chemical Co., USA. Stearylamine (SA) as a positive charge inducer and dicetylphosphate (DP) as a negative charge inducer were obtained from Fluka Chemical Co., Germany. Methanol, chloroform, sodium acetate trihydrate, and glacial acetic acid were purchased from Adwic, El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt. Spectra/Pore® dialysis membrane (12,000–14,000 molecular weight cut-off) was purchased from Spectrum Laboratories Inc., USA. All other reagents were of analytical grade. The commercially available ciprofloxacin otic/ophthalmic solution, Ciprocin® drops, was manufactured by EIPICO Co., Egypt.

2.2. Methods

2.2.1. Preparation of ciprofloxacin transfersomes

Several EAs with different hydrophilic–lipophilic balance (HLB) values (1–>24) were used to prepare ciprofloxacin nano-transfersomal vesicles by TFH technique at PC:EA molar ratio of 5:1 (Abdelbary, 2011), (Table 1). All EAs are nonionic, except sodium cholate and sodium deoxycholate, which are anionic. In brief, PC together with the EA were accurately weighed into a long-necked round-bottom flask and dissolved in 10 mL of chloroform–methanol mixture (1:1). The organic phase was slowly evaporated at 50 °C under vacuum, using a rotary evaporator (Rotavapor, Heidolph VV 2000, Burladingen, Germany) at 90 rpm such that a thin dry film of the components was formed on the inner wall of the flask. The dried thin film was then hydrated with 5 mL of acetate buffer (pH 4.5, buffer strength = 100 mM), containing 25 mg ciprofloxacin, by rotating the flask in a water bath at 50 °C using

Table 1
Entrapment efficiencies (EE%) of transfersomes prepared using different types of EAs.

Formula[a]	EA used in the formulation			EE%[d]
	Name	Molecular weight	HLB	
P L-121	Pluronic L-121	4400[b]	1–7[b]	33.49 ± 9.45
P P-123	Pluronic P-123	5750[b]	7–12[b]	13.76 ± 0.37
P F-127	Pluronic F-127	12600[b]	22[b]	12.89 ± 1.11
P F-68	Pluronic F-68	8400[b]	>24[b]	13.49 ± 0.55
S 65	Span 65	964[b]	2.1[b]	49.51 ± 4.12
S 60	Span 60	431[b]	4.7[b]	59.92 ± 4.68
S 20	Span 20	346[b]	8.6[b]	41.94 ± 4.00
CP-EL	Cremophor EL	2472[c]	12–14[b]	22.82 ± 0.62
CP-RH	Cremophor RH 40	2698[c]	14–16[b]	19.90 ± 2.65
SC	Sodium cholate	430.6[b]	18[b]	64.41 ± 1.91
SDC	Sodium deoxycholate	414.6[b]	16[b]	45.16 ± 7.45

[a] All formulations contained PC and EA at the molar ratio of 5:1, respectively. Total drug content in all of these formulae was ≈100%.
[b] As reported by the manufacturer.
[c] Calculated value.
[d] Each value represents mean ± standard deviation (SD) of three determinations ($n = 3$).