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Enhancement of follicular delivery of finasteride by liposomes and niosomes 1. *In vitro* permeation and *in vivo* deposition studies using hamster flank and ear models

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Abstract

Finasteride is indicated orally in the treatment of androgenetic alopecia and some other pilosebaceous unit (PSU) disorders. We wished to investigate whether topical application of finasteride-containing vesicles (liposomes and niosomes) could enhance drug concentration at the PSU, as compared to finasteride hydroalcoholic solution (HA). Liposomes consisted of phospholipid (dimyristoyl phosphatidylcholine (DMPC) or egg lecithin):cholesterol:dicetylphosphate (8:2:1, mole ratio). Niosomes were comprising non-ionic surfactant (polyoxyethylene alkyl ethers (Brij® series) or sorbitan monopalmitate):cholesterol:dicetylphosphate (7:3:1, mole ratio). Vesicles were prepared by the film hydration technique and characterized with regard to the size, drug entrapment efficiency and gel–liquid transition temperature (T_c). *In vitro* permeation of ^{3}H-finasteride through hamster flank skin was faster from hydroalcoholic solution (0.13 μg/cm^2 h) compared to vesicles (0.025–0.058 μg/cm^2 h). *In vivo* deposition of ^{3}H-finasteride vesicles in hamster ear showed that liquid-state vesicle, i.e. those made of DMPC or Brij97:Brij76 (1:1), were able to deposit 2.1 or 2.3% of the applied dose to the PSU, respectively. This was significantly higher than drug deposition by gel-state vesicles (0.35–0.51%) or HA (0.76%). Both *in vitro* permeation and *in vivo* deposition studies, demonstrated the potentials of liquid-state liposomes and niosomes for successful delivery of finasteride to the PSU.

Keywords: Finasteride deposition in the pilosebaceous units; Niosomes; Liposomes; Skin permeation; Hamster; Follicular drug delivery

1. Introduction

Skin disorders such as acne, seborrhea, hirsutism and androgenetic alopecia are secondary to excess local activity of androgens, more specifically dihydrotestosterone (DHT) in the pilosebaceous unit (PSU). DHT is formed from testosterone via the pivotal enzyme, 5α-reductase. The possibility to target 5α-reductase inhibitors to the PSU to alleviate the disease states associated with or originating within PSU has been implicated in a number of studies (Matias and Orentreich, 1983; Matias et al., 1988; Lieb et al., 1994; Chen et al., 1995).

Finasteride is a known 5α-reductase inhibitor (Tian et al., 1994; Bull et al., 1996) expected to be pharmacologically active in the pilosebaceous unit (Chen et al., 1995, 1996). There have been several clinical studies on the efficacy of oral finasteride in the treatment of seborrhoea (Chen et al., 1996), female hirsutism (Moghetti et al., 1994; Wong et al., 1995) and androgenetic alopecia (Dallob et al., 1994; Chen et al., 1996; Kaufman et al., 1998; Drake et al., 1999; Meidan and Touitou, 2001; Shapiro and Kaufman, 2003). Finasteride also has been used topically to treat androgenetic alopecia (Mazzarella et al., 1997).

In recent years, many attempts have been made to enhance drug deposition in the PSU using delivery systems such as nanoemulsions (Lieb et al., 1992; Wu et al., 2001), solid lipid nanoparticles (Maia et al., 2002), low-molecular weight dextrans (Lieb et al., 1995), microspheres (Rolland et al., 1993; Sumian et al., 1999; Toll et al., 2004), iontophoresis (Burnette and Ongpipattanakul, 1988; Cullander and Guy, 1991; Green

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et al., 1991) and niosomes or liposomes (Balsari et al., 1994; Lieb et al., 1994; Lauer et al., 1995, 1996; Niemiec et al., 1997; Bernard et al., 1997; Weiner, 1998; Toll et al., 2004). Experiments with liposomes or niosomes in the Syrian hamster ear model have demonstrated that the vesicles containing carboxyfluorescein (Lieb et al., 1992), cimetidine (Lieb et al., 1994) and peptides (Niemiec et al., 1995) delivered higher drug concentrations into the sebaceous glands as compared to conventional formulations. High drug localization into the hair follicles of histocultured mouse have been also reported for calcein (Li et al., 1992), melanin (Li et al., 1993b) and DNA (Li et al., 1993a) containing liposomes.

Liposomes and niosomes, made of phospholipids and non-ionic surfactants, respectively, have several advantages over conventional non-vesicle formulations. A major advantage lies in their amphipathic nature, which allows incorporation of a wide variety of hydrophilic and hydrophobic drugs (Uchegbu and Vyas, 1998). They may serve as a solubilizing matrix, as a local depot for sustained release or permeation enhancers of dermally active compounds or as a rate limiting membrane for the modulation of systemic absorption of drugs via the skin (Touitou et al., 1994; Agarwal et al., 2001; Manosroi et al., 2003).

Liposome and niosome characteristics such as lamellarity, lipid composition and structure (El Maghraby et al., 1999), surface charge and size (Plessis et al., 1994), as well as physicochemical nature of the drug itself (Lauer, 1999; Grams and Bouwstra, 2002; Ogiso et al., 2002; Grams et al., 2003) may affect of follicular deposition of drugs. Vesicle–skin interactions and the therapeutic efficacy of vesicular formulation can be strongly affected, in particular, by phase state (Bouwstra et al., 1997; Uchegbu and Vyas, 1998) and elasticity (van den Bergh et al., 1999; El Maghraby et al., 1999; Trotta et al., 2002); bilayers in the liquid-state are not closely packed as in the gel state, and thus can penetrate within skin strata more easily.

In this study, we sought to evaluate the effects of composition and physical state of vesicles on the extent of finasteride permeation through and deposition into the different strata of the hamster flank and ear skin.

2. Materials and methods

2.1. Materials

Non-ionic surfactants: Brij52 (polyoxyethylene 2 cetyl ether), Brij72 (polyoxyethylene 2 stearyl ether), Brij76 (polyoxyethylene 10 stearyl ether), Brij97 (polyoxyethylene 10 oleyl ether), Span40 (sorbitan monopalmitate) and cholesterol (chol) were purchased from Fluka (Switzerland). Dicetyl phosphate (DCP) was obtained from sigma (USA). Dimyristoyl phosphatidylcholine (DMPC) was from Nippon Fine Chemicals (Japan). ^{3}H-finasteride, having the specific activity of 25 Ci/mmol, was purchased from American Radiochemicals (ARC, USA) and finasteride was from Cipla Inc. (India). Clobazam was kindly provided by Hakim Pharmaceuticals (Tehran, Iran). Hyamine hydroxide (ICN Biochemical) was used as a tissue solubilizer. Ready Value®, Ready organic® and Ready Protein+® scintillation cocktail were obtained from Beckman (USA). Sephadex G-25 coarse was bought from Pharmacia LKB (Uppsala, Sweden). Egg lecithin, acetonitrile LC grade and methanol LC grade were obtained from Merck (Germany). All other chemicals and solvents were of analytical grade. HPLC-grade de-ionised water was produced using Direct-Q^{TM} (Millipore, France).

2.2. Animals

Male adult Golden Syrian hamsters (weighing 80–110 g), 10–12 weeks of age, were purchased from Pasteur Institute (Tehran, Iran). The animals were housed one per cage in plastic boxes on sawdust with tap water. The animals were housed at a photoperiod of about 14-h light and 10-h darkness and ambient temperature, for at least 2 weeks prior to the experiments. The skin overlying the flank organs was closely shaved with an electric hair clipper. The experiments using animals were approved by the university's ethics committee.

2.3. Vesicle preparation

Multilamellar vesicles (MLVs) were prepared by the film formation method as reported by Bangham et al. (1965) and Baillie et al. (1985) with some modifications. Lipid components of niosomes comprising surfactant (one or mixture of two):chol:DCP (7:3:1, m.r.) or liposomes consisting phospholipid (egg lecithin or DMPC):chol:DCP (8:2:1, m.r.) were dissolved in chloroform:methanol (2:1 v/v). The total lipid concentration was 30 mM. Finasteride solution in the same solvent (0.53 mM) was spiked with ^{3}H-finasteride at 8 μCi/ml and added to the lipid solution in a round bottom flask. The solvents were removed using a rotary evaporator at a reduced pressure. The dried thin film was hydrated with PBS (pH 7.4) for 30 min at 10 °C above the phase transition temperature (T_c) of the amphiphiles while shaking. The dispersion was left for 4 h at room temperature (RT) to complete hydration and then stored at 4 °C overnight before use.

2.4. Determination of finasteride entrapment efficiency (EE%)

Finasteride-containing MLVs were separated from unentrapped drug by size exclusion chromatography (SEC) using Sephadex G-25 coarse gel and pH 7.4 PBS as eluting solvent. Empty liposomes/niosomes, extruded through 400 nm polycarbonate membrane (Nucleopore, Canada) were used to presaturate the column. Typically, a 100 μl sample of finasteride–nisomes was loaded on the column, then PBS was eluted at a flowing rate of 1 ml/min. The turbid fraction *containing vesicles* was collected and dissolved in methanol:PBS (1:1 v/v). The finasteride content of the vesicles was then determined by HPLC using C_{18} μBondapack Waters column (4.6 mm × 250 mm, 10 μm); the mobile phase consisted of 40:60 (v/v) acetonitrile:15 mM KH_2PO_4 (pH 4.5) and was pumped at a rate of 1.4 ml/min. Clobazam was used as internal standard. Finasteride entrapment efficiency is expressed as percent of initial amount of drug which was entrapped within