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Formulation and in vitro assessment of minoxidil niosomes for enhanced skin delivery

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ABSTRACT

Niosomes have been reported as a possible approach to improve the low skin penetration and bioavailability characteristics shown by conventional topical vehicle for minoxidil. Niosomes formed from polyoxyethylene alkyl ethers (Brij™) or sorbitan monoesters (Span™) with cholesterol molar ratios of 0, 1 and 1.5 were prepared with varying drug amount 20–50 mg using thin film-hydration method. The prepared systems were characterized for entrapment efficiency, particle size, zeta potential and stability. Skin permeation studies were performed using static vertical diffusion Franz cells and hairless mouse skin treated with either niosomes, control minoxidil solution (propylene glycol–water–ethanol at 20:30:50, v/v/v) or a leading topical minoxidil commercial formulation (Minoxyl). The results showed that the type of surfactant, cholesterol and incorporated amount of drug altered the entrapment efficiency of niosomes. Higher entrapment efficiency was obtained with the niosomes prepared from Span 60 and cholesterol at 1:1 molar ratio using 25 mg drug. Niosomal formulations have shown a fairly high retention of minoxidil inside the vesicles (80%) at refrigerated temperature up to a period of 3 months. It was observed that both dialyzed and non-dialyzed niosomal formulations (1.03 ± 0.18 to 19.41 ± 4.04%) enhanced the percentage of dose accumulated in the skin compared to commercial and control formulations (0.11 ± 0.03 to 0.48 ± 0.17%) except dialyzed Span 60 niosomes. The greatest skin accumulation was always obtained with non-dialyzed vesicular formulations. Our results suggest that these niosomal formulations could constitute a promising approach for the topical delivery of minoxidil in hair loss treatment.

1. Introduction

Minoxidil, a pyrimidine derivative (2, 4-diamino-6-piperidinopyrimidine-3-oxide, Fig. 1), is the only topical medical treatment with proven efficacy for the treatment of Androgenetic alopecia (AGA). AGA is hereditary and is the progressive, androgen-dependent thinning of scalp hair, which follows a definite pattern. The US Food and Drug Administration approved treatments for AGA are oral finasteride at a dose of 1 mg per day and topical solutions of 2 and 5% minoxidil (Price, 1999). Little is known of the effect of minoxidil on normal human hair growth and studies have been limited mainly to the response of androgenetic alopecia to topical minoxidil. Recently, Han et al., 2004 reported that minoxidil stimulates hair growth in human by prolonging anagen through proliferative (by activating both ERK and Akt) and antiapoptotic (by increasing the ratio of Bcl-2/Bax) effects on dermal papilla cells (DPCs) of human hair follicles. Minoxidil have been reported for its poor skin penetration ability, which limits minoxidil usefulness as a potent drug in the use of hair growth treatment.

Minoxidil is poorly soluble in water and most of the water-immiscible organic solvents such as chloroform. Therefore, it has been formulated for topical use in an ethanol-based solution containing ethanol, propylene glycol and water (Tata et al., 1995). One drawback of ethanol-based formulations is the tendency of the minoxidil to revert to an insoluble crystalline form when applied to the skin, as the ethanol solvent evaporates. Whether due to the tendency of the drug to crystallize or other factors, the minoxidil formulation shows relatively inefficient uptake by the skin. Further, evaporation of ethanol, when the formulation is applied to the skin, leaves a viscous propylene glycol/water residue which may be objectionable to many users. Moreover, typical side effects of the topical treatment with ethanol-based minoxidil formulations

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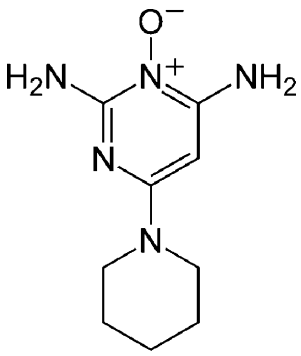

Fig. 1. Chemical structure of minoxidil (3-hydroxy-2-imino-6-(1-piperidyl) pyrimidin-4-amine).

include irritative dermatitis going along with pruritus, erythema, scaling and dryness occur at the onset of therapy (Williams and Barry, 2004). In some cases, allergic contact dermatitis or exacerbation of seborrhoic dermatitis has been reported. While most of the patients with allergic contact dermatitis described in the literature showed a positive sensitization to the vehicle substance propylene glycol evaluated by patch testing, reactions to the active ingredient minoxidil are rare (Hagemann et al., 2005). Since most of the conventional topical minoxidil formulations consist of propylene glycol–water–ethanol solution, to minimize the side effects and to improve the therapeutic efficiency, new dermatological preparations with free of organic solvents and propylene glycol are required.

Vesicular system, both liposomes and niosomes are uni- or multilamellar spheroidal structures composed of amphiphilic molecules assembled into bilayers. They are considered primitive cell models, cell-like bioreactors and matrices for bioencapsulation. In the recent years, nonionic surfactant vesicles known as niosomes received great attention as an alternative potential drug delivery system to conventional liposomes. Moreover, compared to phospholipid vesicles, niosomes offer higher chemical and physical stability (Vora et al., 1998) with lower cost and greater availability of surfactant classes (Manconi et al., 2006). Niosomes have been reported to enhance the residence time of drugs in the stratum corneum and epidermis, while reducing the systemic absorption of the drug and improve penetration of the trapped substances across the skin. In addition, these systems have been reported to decrease side effects and to give a considerable drug release (Schreier and Bouwstra, 1994). They are thought to improve the horny layer properties both by reducing transepidermal water loss and by increasing smoothness via replenishing lost skin lipids (Junginger et al., 1991). Moreover, it has been reported in several studies that compared to conventional dosage forms, vesicular formulations exhibited an enhanced cutaneous drug bioavailability (Manconi et al., 2006; Mura et al., 2007).

In this work, the effects of minoxidil entrapped niosomes on the drug penetration in hairless mouse skin were investigated by in vitro permeation experiments, and compared with those of control minoxidil solution 5 mg/mL (propylene glycol–water–ethanol at 20:30:50, v/v/v) and a leading commercial topical formulation "Minoxyl™" containing 5% minoxidil and 1% dexpentanol (Hyundai Pharma Ltd. South Korea). Niosomal formulations were prepared by thin film-hydration (TFH) method using cholesterol and nonionic surfactants polyoxyethylene 2 cetyl ether (Brij 52), polyethylene glycol octadecyl ether (Brij 76), and sorbitan monoesters (Span 20, Span 40, Span 60 and Span 80). This paper focuses on the properties of thin film-hydrated niosomes as potential new minoxidil carriers for effective skin delivery.

Table 1
Composition of vesicles as minoxidil carriers (mg/batch).

Components	HLB	*Tc* (°C)	1:1 (surf:chol) (mg)	1:1.5 (surf:chol) (mg)
Cholesterol Surfactants			38.6	57.9
Brij 52	5.3	32.5	33	33
Brij 76	12.4	34	71.1	71.1
Span 20	8.6	16	34.6	34.6
Span 40	5.7	42	40.2	40.2
Span 60	4.7	53	43.6	43.6

*In all the formulations, DCP 8.2 mg used.
*Minoxidil used (20, 25, 30, 40 and 50 mg).
*Gel–liquid transition temperature (*Tc*).

2. Materials and methods

2.1. Chemicals

Cholesterol, dicetyl phosphate (DCP), polyoxyethylene 2 cetyl ether (Brij 52) and polyethylene glycol octadecyl ether (Brij 76) were purchased from Sigma Aldrich (St. Louis, USA). Minoxidil (MW 209.25, 99% purity) was kindly provided by Hunmi pharmaceutical company (Seoul, South Korea). Methanol and ethanol were supplied by Burdick and Jackson (Ulsan, South Korea). Span 20 and Span 80 were purchased from Junsei chemicals Co. Ltd. (Tokyo, Japan). Span 40 and Span 60 were obtained from Yakuri pure chemicals Co. Ltd. (Kyoto, Japan). Propylene glycol and chloroform were supplied by D. C. chemical Co. Ltd. (Seoul, South Korea). A 5% Minoxyl® topical solution sold by Hyundai pharm. Co. Ltd. (Seoul, South Korea) was purchased from a local market. All other materials and solvents used in this study were of analytical grade.

2.2. Vesicle preparation

Niosome preparation: Minoxidil niosomes were prepared using thin film-hydration method. Accurately weighed quantities of the surfactant (Brij™ or Span™)) and cholesterol in different molar ratios, viz. 1:0, 1:1 and 1:1.5, were dissolved in 8 mL of chloroform:methanol mixture (2:1, v/v) in a round-bottom flask (Agarwal et al., 2001). Afterwards, minoxidil and DCP dissolved in 5 mL of chloroform:methanol mixture (2:1, v/v) was added to the lipid solution (Table 1). The organic solvents were removed under vacuum in a rotary evaporator at 40 °C for 20 min to form a thin film on the wall of the flask, and kept in a desiccator under vacuum for 2 h to ensure total removal of trace solvents. After removal of the last trace of organic solvents, hydration of the surfactant film was carried out using 10 mL of distilled water at 55 °C, which is above the gel–liquid transition temperature (*Tc*) of sorbitan monoesters and polyoxyethylene alkyl ether surfactants (Abbas et al., 2007; Azeem et al., 2008). The resulting niosomal suspension was mechanically shaken for 1 h using a horizontal mechanical shaking water bath at 55 °C. Then, the vesicle suspension was sonicated (Branson 5510R-DTH, USA) in 3 cycles of 1 min "on" and 1 min "off" leading to the formation of multilamellar niosomes. The niosomal suspension was left to mature overnight at 4 °C and stored at refrigerator temperature for further studies.

2.3. Content of drug in vesicles

The prepared minoxidil niosomes were separated from unentrapped minoxidil by exhaustive dialysis (Mura et al., 2007) for 3 h in distilled water using Spectra-Por® membranes (12,000–14,000 cut-off, Spectrum laboratories, Inc., USA). The amount of entrapped minoxidil was determined by lysis of the dialyzed vesicles with absolute ethanol. A 100 μL sample of niosomes was mixed with 5 mL of absolute ethanol and covered well with parafilm to pre-