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Solid lipid nanoparticles suspension versus commercial solutions for dermal delivery of minoxidil

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ABSTRACT

Solid lipid nanoparticles have been reported as possible carrier for skin drug delivery. Solid lipid nanoparticles are produced from biocompatible and biodegradable lipids. Solid lipid nanoparticles made of semi-synthetic triglycerides stabilized with a mixture of polysorbate and sorbitan oleate were loaded with 5% of minoxidil. The prepared systems were characterized for particle size, pH and drug content. *Ex vivo* skin penetration studies were performed using Franz-type glass diffusion cells and pig ear skin. *Ex vivo* skin corrosion studies were realized with a method derived from the Corrositex® test. Solid lipid nanoparticles suspensions were compared to commercial solutions in terms of skin penetration and skin corrosion. Solid lipid nanoparticles suspensions have been shown as efficient as commercial solutions for skin penetration; and were non-corrosive while commercial solutions presented a corrosive potential. Solid lipid nanoparticles suspensions would constitute a promising formulation for hair loss treatment.

1. Introduction

Androgenic alopecia is a common form of hair loss in both men and women. Minoxidil, a pyridine-derivative, is widely used for the treatment of androgenic alopecia (Atrux-Tallau et al., 2009). Commercial products containing minoxidil are usually solutions with high percentage of ethyl alcohol and/or propylene glycol (Tata et al., 1995). Twice-daily applications are recommended as proper use. However repeated applications of high ethyl alcohol and/or propylene glycol content products lead to severe adverse effects (e.g., scalp dryness, irritation, burning, redness, allergic contact dermatitis) (Aronson, 2006; Pavithran, 1993; Wagner and Kenreigh, 2007). Since most of the products containing minoxidil available on the market consist of ethyl alcohol – propylene glycol – water solutions, new dermatological formulations free of organic solvents are needed to minimize adverse effects and optimize androgenic alopecia treatment. Recent work proposed liposomes or niosomes as alternative drug delivery systems. Mura et al. (2007) showed alcohol-free liposomal formulations potential as minoxidil topical delivery in hair loss treatment. Balakrishnan et al. (2009) suggested that niosomal formulations of minoxidil have a good potential for drug cutaneous targeting with regards to process variables. Mura et al. (2009) studied penetration enhancer-containing vesicles as carriers for cutaneous delivery of minoxidil and showed that association of soy Phosphatidylcholine and Transcutol® does not improve minoxidil diffusion through skin layers (Mura et al., 2011). Nanoparticles and microparticles have been largely developed for skin drug delivery. Skin provides a natural barrier against exogenous aggressions and particles penetration. However therapeutic nanoparticles and microparticles can be delivered in diseased skin and to hair follicles openings (Knorr et al., 2009; Lademann et al., 2007; Prow et al., 2011). Solid Lipid Nanoparticles (SLN) are composed of solid lipid cores (i.e., lipids solid at room temperature) stabilized by surfactant(s) in water suspension. The use of triglycerides in SLN formulations is an advantage in terms of toxicity (Traul et al., 2000). SLN are easily produced by melt emulsification followed by a homogenization step either with high pressure homogenizer or ultrasonic processor (Padois et al., 2009). SLN have been largely studied as potential carriers for topical drug application in skin diseases (Mehnert and Mäder, 2001; Müller et al., 2000,

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Table 1
SLN suspension composition.

Components	Content (g)
Minoxidil	5
Suppocire® NAI50	10
Montane® 80PHA	6
Montanox® 20PHA	4
Phosal® 50PG	4
Water	71

2002, 2007; Saupe and Rades, 2006; Schäfer-Korting et al., 2007; Souto and Doktorovová, 2009).

In the present work, a new SLN suspension containing 5% of minoxidil is proposed. Drug deposition in the different skin layers was determined *ex vivo* using Franz-type glass diffusion cells and compared to commercial products. Furthermore *ex vivo* skin corrosion studies were conducted in order to evaluate and compare the corrosive factor of new SLN suspension and commercial products. This paper focuses on the new SLN suspension potential as safe minoxidil carrier for androgenic alopecia treatment.

2. Materials and methods

2.1. Materials

Semi-synthetic triglycerides (Suppocire® NAI50) were kindly obtained from Gattefossé (Saint Priest, France). Polysorbate and sorbitan oleate (Montanox® 20PHA and Montane® 80PHA) were a gift from Seppic (Castres, France). Phosphatidylcholine (Phosal® 50PG) was obtained from Welding Pharma (Lyon, France). Minoxidil was a gift from Pierre Fabre (Ramonville Sainte Agne, France). Ovalbumin, NaCl, sulforhodamine B and dioctyl sulfosuccinate sodium salt were purchased from Sigma Aldrich (Saint Quentin Fallavier, France). Acetic acid, perchloric acid, nitric acid and methanol were purchased from Carlo Erba (Val de Reuil, France). All solvents were of analytical grade. Alopexy 5% (Pierre Fabre Dermatologie, Boulogne, France) and Minoxidil Bailleul 5% (Laboratoire Bailleul-Biorga, Villeneuve la Garenne, France) were purchased in a local pharmacy.

2.2. SLN suspension preparation

SLN suspension composition is reported in Table 1 (Padois et al., 2009). Commercial solutions compositions are reported in Table 2. SLN suspensions were prepared according to procedure described in the patent FR0952059 (Padois et al., 2009). Suppocire® NAI50, Montane® 80PHA, Montanox® 20PHA and Phosal® 50PG were mixed and heated at 40 ± 2 °C. Minoxidil was added to the mixture at 40 ± 2 °C under stirring at 100 rpm. Lipid–drug mixture was maintained under stirring at 40 ± 2 °C during 5 h. Water was heated at 40 ± 2 °C and added to the lipid–drug mixture under stirring at 500 rpm. Stirring was maintained during 1 h at 40 ± 2 °C. The obtained pre-emulsion was homogenized at the temperature of the melt using a high pressure homogenizer Emulsiflex® C5 (HHP) (Avestin GmbH, Mannheim, Germany) applying 100,000 kPa and three homogenization cycles.

Table 2
Commercial solutions compositions (g/100 mL).

Components	Alopexy 5%	Minoxidil Bailleul 5%
Minoxidil	5	5
Ethyl alcohol (96%)	25	58.6
Propylene glycol	50	20
Water	QS 100 mL	QS 100 mL

2.3. SLN suspension characterization

Photon correlation spectroscopy (PCS), performed using a Zetamaster® (Malvern Instruments, France), was used to assess the mean particle size (*z*-diameter) and the polydispersity index (PI). Data were fitted by the Contin method. Zeta potential (ξ) was measured as the particle electrophoretic mobility means of laser microelectrophoresis. A pH-meter Cyberscan® pH 110 (Eutech Instruments, Singapore) was used to determine the tested products pH. All measurements were made at 20 ± 2 °C.

2.4. Minoxidil content and drug loading

Minoxidil content (MC%) was determined by High Performance Liquid Chromatography (HPLC Agilent 1200 Series, Massy, France) directly by diluting the commercial solutions into the mobile phase or after disruption of SLN in the mobile phase. HPLC method was adapted from Asmus et al. (1984). Minoxidil content is expressed in gram of minoxidil per 100 g of sample. Analysis was carried out by reverse-phase absorption chromatography using a column Kinetex® (2.6 μm, 100 Å, C18, 4.6 mm × 100 mm) (Phenomenex, Le Pecq, France). The mobile phase was a mixture of water, methanol, acetic acid and dioctyl sulfosuccinate sodium salt (300 mL, 700 mL, 10 mL and 3 g, respectively). Mobile phase pH was adjusted to 3.0 with perchloric acid. The flow rate was 0.7 mL/min. The sample volume injected is 20 μL. Detection was performed at 254 nm. For concentrations from 1.56 to 100 μg/mL, chromatograms were linear with a factor correlation of 0.999. The method reproducibility was tested for 3 concentrations (1.56 μg/mL, 50 μg/mL and 100 μg/mL). The coefficient of variation was lower than 2.5%. The limit of quantification (LOQ) and the limit of detection (LOD) for chromatographic determination of minoxidil were calculated from the calibration curve (Eqs. (1) and (2)) (MacDougall and Crummett, 1980). LOQ was 0.37 μg/mL and LOD was 0.11 μg/mL.

$$\mathrm{LOQ} = 10 \times \left(\frac{\sigma b}{a}\right) \quad (1)$$

$$\mathrm{LOD} = 3 \times \left(\frac{\sigma b}{a}\right) \quad (2)$$

with a is the regression line gradient and σb is the intercept point deviation.

SLN drug loading (DL%) was expressed as the percentage of the amount of minoxidil initially used taking into account the water loss by evaporation during the SLN suspension preparation.

2.5. Ex vivo skin penetration studies

Pig ears were obtained from a local slaughterhouse. The skin was kept frozen until use. Before use, skin was briefly washed under tap water and full-thickness skin was removed from the dorsal side of the pig ear. Skin punches were used for *ex vivo* penetration studies carried out with Franz-type glass diffusion cells. These cells consist of two compartments with the skin clamped between the donor and receiver chambers, dermal side down. The receiver chamber was filled with a receptor phase consisting of water with 0.9% NaCl and 3% ovalbumin (Mura et al., 2007). The receptor phase was constantly stirred with a magnetic bar and thermostated at 37 ± 1 °C (resulting in a skin surface temperature of 32 ± 1 °C) throughout the experiments. A determined volume of studied product was placed in the donor chamber onto the stratum corneum of the porcine skin, in non-occlusive conditions. The diffusion area was 0.785 cm^2. At the end of the experiment (24 h), the skin surface was washed with 2 × 1 mL of distilled water to remove the residual donor sample. The receptor phase was removed and the Franz-type glass diffusion cells were dismantled. The skin

exposed to the sample (0.785 cm^2) was punched out. Epidermis was separated from dermis by heat treatment. Both were grinded into HPLC mobile phase. Tissue suspensions were centrifuged for 15 min at 12,000 rpm, then the supernatants were filtered (0.45 μm) and assayed for the minoxidil content by HPLC as described above. The experiments were conducted at least in triplicate.

2.6. Ex vivo skin corrosion studies

The human skin is exposed to a great amount of different chemicals that need to be classified according to their capacity to harm the skin (Gordon et al., 1994; Groeber et al., 2011; Macfarlane et al., 2009). The product is defined as corrosive if the skin is irreversibly damaged (Roguet, 1999), whereas the product is defined as irritative, if the skin is reversibly altered (Welss et al., 2004). Assays were developed based on tissue-engineered living skin substitutes (Perkins et al., 1996). In order to determine the corrosive potential of SLN suspensions and commercial products, an *ex vivo* method has been developed derived from the Corrositex® test (Robinson et al., 2002; Stobbe et al., 2003). Corrosive substances are able to destroy the epidermis proteins and lead to a color shift in an underlying chemical detection liquid. Corrosive potential of formulations was determined on pig ear skin. 37% nitric acid and 0.9% NaCl solutions were used as positive and negative control, respectively. Skin punches were prepared and clamped on Franz-type glass diffusion cells as explained in Section 2.5. 200 μL of 37% nitric acid solution, 0.9% NaCl solution or studied sample was deposited onto the epidermis. After 15 min, the 200 μL was removed. Epidermis was washed with 2 × 1 mL of distilled water to remove the residual sample. 1 mL of sulforhodamine B (skin proteins labeling dye) was deposited onto the epidermis. After 15 min, the 1 mL of sulforhodamine B was removed. Then epidermis was washed with 1 mL of distilled water. The absorbance (Abs) of the washing water was measured with a spectrophotometer Graphicord UV-240 (Shimadzu, Kyoto, Japan) at 265.5 nm. The corrosive factor F was calculated with the Eq. (3).

$$F = \frac{(\text{sample Abs} - 0.9\% \ \text{NaCl solution Abs})}{(0.9\% \ \text{NaCl solution Abs})} \quad (3)$$

If $F > 0$, then the sample is non corrosive. If $F < 0$, then the sample is corrosive.

The experiments were conducted six times.

2.7. Statistical analysis of data

Data analysis was carried out with the software R 2.12.2. Results are expressed as the mean ± standard deviation of at least three experiments. Statistically significant difference was determined using non-parametric tests: Wilcoxon test or Kruskal–Wallis test. Significance was tested with P at the 0.05 level of probability.

3. Results and discussion

Minoxidil is the most commonly used drug for androgenic alopecia treatment. Its mechanism of action remains unknown but the hair follicle has been regarded as the site of action (Messenger and Rundegren, 2004). A recent report identified ATP-channel responsive to minoxidil located in human follicular dermal papillae (Grice et al., 2010). Blume-Peytavi et al. (2010) demonstrated the high importance of the follicular pathway penetration pathway for topically applied minoxidil foam. Thus SLN particle size is important in order to target the hair follicles. Within the hair follicles, different target sites of interest have been defined (Patzelt et al., 2008). The sebaceous gland is of particular interest and associates with the aetiology of androgenic alopecia (Meidan and Touitou, 2001). As reported in Table 3, the mean particle size of the SLN was about 190 nm with a narrow distribution (PI < 0.20). These z-diameter values are favorable for minoxidil delivery to the sebaceous gland. In fact, Patzelt et al. (2011) had recently showed that particles of this size range are qualified for penetrating into the sebaceous gland region of porcine terminal hair follicles. They expected results to be similar in human terminal hair follicles because of the similarity in size of porcine and human terminal hair follicles. Zeta potential values of SLN suspensions were just below the critical value of −30 mV, which is required for a good physical stability (Freitas and Müller, 1998). However neither modification in SLN particle size nor SLN suspension gelation were observed over a period of 6 weeks. Thus a zeta potential just below −30 mV in combination with non-ionic surfactants stabilization is sufficient to physically stabilize SLN suspensions. The total amount of minoxidil was similar for all the formulations and the same variation range was observed between SLN suspension batches and between commercial solution batches. The drug loading was the same for the SLN suspension batches (DL% ≅94%). As a consequence of minoxidil solubility, a different partition of the drug between the water phase and the SLN may be supposed. At pH = 6.50, Minoxidil log D = 0.44. Thus, about 75% of the minoxidil should be encapsulated in the SLN. It is anticipated that the product would display a biphasic release profile: a burst effect due to the minoxidil contained in the water phase and a prolonged released due to the minoxidil encapsulation into the SLN (Müller et al., 2000).

In the present study, *ex vivo* penetration studies were carried out with Franz-type glass diffusion cells in order to compare SLN suspension with commercial products. Porcine skin was treated with 2 different amounts of formulation: 200 μL in order to have an excess of formulation onto the skin, or 10 μL corresponding to the usual treatment of androgenic alopecia with formulations containing 5% of minoxidil.

In the present study, permeation of minoxidil through porcine skin into the receiver chamber of Franz-type glass diffusion cell was never detected, taking into account the LOD of HPLC system. In contrast, minoxidil penetration is seen through excised human epidermis with a lag time of 6 h suggests that the dermis is leading to a longer lag time for penetration into the receiver of our pig skin studies that is not detected at 24 h (Grice et al., 2010). Minoxidil contents in epidermis and dermis are reported in Table 4. Skin penetration values were expressed as drug amount per tissue weight. High standard deviations can be noticed in Table 4. As reported by Wester and Maibach (1989), high individual and regional variation exists for *in vivo* and *in vitro* percutaneous absorption studies in human as well as in animal. Penetration experiments with porcine skin showed that the SLN suspension penetration into skin layers was statistically non-different to commercial products for formulation applied in excess (200 μL) as well as usual androgenic alopecia treatment (10 μL). Mura et al. (2011) studied the minoxidil topical delivery. During their experiments, skin surface was treated with the same minoxidil amount as our experiments (200 μL of formulation ≅1.5 mg/cm^2 of minoxidil) but during only 8 h. Their results highlighted that minoxidil was mainly distributed in the outermost skin layers and only a small amount of minoxidil reached the dermis. Our results showed that after 24 h of treatment, the minoxidil was distributed identically in the epidermis and the dermis.

As explained by Agache (2004), the skin surface is an ecosystem in equilibrium with precise characteristics. The value of the skin pH is between 4.2 and 6.1. Applied products with pH out of this range could lead to skin surface disorder. pH of the SLN suspensions and tested commercial products are reported in Table 4. pH of SLN suspensions was close to skin pH whereas pH of studied commercial products was over 7.00. With such difference in pH value, different cutaneous reactions can be expected during long-term treatment.

Repeated applications of ethyl alcohol – propylene glycol – water minoxidil-based solutions resulted in typical adverse effects

Table 3
Characterization of minoxidil formulations.

Formulation	MC (%)	pH	*z*-Diameter (nm)	PI	ξ (mV)	DL (%)
SLN suspension batch 1	6.56 ± 0.02	6.50 ± 0.02	189 ± 2	0.15 ± 0.04	−26 ± 2	94.46 ± 1.41
SLN suspension batch 2	8.76 ± 0.03	6.25 ± 0.04	191 ± 2	0.19 ± 0.02	−28 ± 2	94.61 ± 1.88
Alopexy 5% batch 1	6.86 ± 0.02	8.84 ± 0.06	–	–	–	
Alopexy 5% batch 2	8.99 ± 0.02	8.02 ± 0.03	–	–	–	
Minoxidil Bailleul 5%	7.26 ± 0.03	8.62 ± 0.06	–	–	–	

Table 4
Determination of minoxidil penetration into porcine skin.

Formulation	Volume on skin (μL)	Minoxidil content (μg/g)		
		Epidermis	Dermis	Total
SLN suspension batch 1	200	662 ± 376	581 ± 339	1243 ± 534*
Alopexy 5% batch 1	200	416 ± 146	387 ± 242	803 ± 141*
SLN suspension batch 2	10	915 ± 350	181 ± 88	1096 ± 420*
Alopexy 5% batch 2	10	796 ± 84	535 ± 93	1331 ± 162*
Minoxidil Bailleul 5%	10	392 ± 82	349 ± 250	741 ± 328*

* $P > 0.05$.

including irritative dermatitis going along with dryness, erythema, and desquamation; implying treatment failure. Golla et al. (2009) developed a skin irritation quantitative structure–property relationship model based on rabbit Draize test data which classified ethanol as irritant chemical compound.

Therefore it is important to investigate the corrosive factor of formulations available for cutaneous application. *Ex vivo* skin corrosion studies results are presented in Table 5. The SLN suspension was non-corrosive while the different tested commercial products exhibited a corrosive potential. Statistical analysis confirmed that the corrosive factor of the SLN suspensions are different from 0 and >0; and that the corrosive factor of the commercial tested products are different from 0 and <0. Moreover statistical analysis attested that there is a difference between the corrosivity of the studied formulations. This corrosive factor of commercial products can explain the low treatment compliance.

In the literature, many studies highlighted that active substances can be encapsulated into SLN in order to decrease and eliminate skin irritation. SLN based tretinoin gel were compared to marketed tretinoin emulsion and the skin irritation studies carried out on rabbits indicated SLN potential in improving skin tolerability of tretinoin (Mandawgade and Patravale, 2008; Shah et al., 2007). As well, retinoic acid marketed emulsions were compared to retinoic acid loaded SLN formulations and skin irritation studies showed that the encapsulation of retinoic acid into SLN resulted in reduced skin irritation (Castro et al., 2009). Furthermore, retinoic acid loaded SLN have shown a potential for drug controlled release. Küchler et al. (2010) studied the influence of morphine loaded SLN on wound healing. These SLN were shown to be less cytotoxic, less irritative and to accelerate wound closure. Results presented in this paper for new minoxidil loaded SLN suspension were likely as for adverse effects decrease. This novel SLN formulation represents a promising alternative for topical treatment of androgenic alopecia with minoxidil.

Table 5
Corrosive factor of the SLN suspensions and commercial products.

Formulation	*F*
37% Nitric acid solution	−0.589 ± 0.173
SLN suspension batch 1	0.534 ± 0.087*,◊
SLN suspension batch 2	0.402 ± 0.136*,◊
Alopexy 5% batch 1	−0.309 ± 0.033♦,◊
Alopexy 5% batch 2	−0.284 ± 0.095♦,◊
Minoxidil Bailleul 5%	−0.118 ± 0.109♦,◊

* $P < 0.05$ and hypothesis >0 is true.
♦ $P < 0.05$ and hypothesis <0 is true.
◊ $P < 0.05$.

4. Conclusion

In the present study, new SLN suspension containing minoxidil was compared to commercial products. SLN diameter was suitable for hair follicles targeting which has been reported as the minoxidil site of action. SLN suspensions *ex vivo* skin penetration has been shown as efficient as tested commercial products. The present work reported a huge difference between SLN suspensions and commercial products in terms of skin corrosion. SLN suspensions, formulated using physiological lipids with solvent-free process, were shown to be totally non-corrosive. Such a formulation should increase the patient compliance.

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