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Ethosomes: An Exciting and Promising Alcoholic Carrier System for Treating Androgenic Alopecia

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Additional information is available at the end of the chapter

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Abstract

Androgenetic alopecia (male-pattern hair loss) is characterized by the deposition of dihydrotestosterone at the pilosebaceous unit of the scalp. Oral administration of drugs (like finasteride) which can reverse androgenic alopecia causes undesired effects to the body. Targeting these drugs directly to the pilosebaceous unit of the scalp will enhance the pharmacological response at the desired site by reducing undesired systemic side effects. This chapter discusses about ethosomes, a specially tailored ethanolic vesicular carriers which can efficiently deliver various drugs with different physicochemical properties to and through the skin. The unique characteristics of the ethosomal carriers, their composition, preparation methods, and the mechanism of permeation, safety, and practical experience (finasteride and herbal extracts) have been discussed in detail.

Keywords: androgenic alopecia, ethosomes, finasteride, nanoparticles

1. Androgenic alopecia

Hair growth in the scalp is an androgen-dependent process. Many androgens target tissues in scalp hair follicles and are more responsive to dihydrotestosterone than testosterone. Two types of 5α-reductase enzymes, which convert testosterone to dihydrotestosterone, are present in humans. Scalp skin contains type 1 5α-reductase in the sebaceous glands and type 2 5α-reductase in the dermal papillae of hair follicles and connective tissue sheaths. Male-pattern hair loss, also referred to as androgenic alopecia, is a type of hair loss that occurs due to shrinkage of hair follicles by the influence of androgenic hormones. Type 2 5α-reductase promotes conversion of testosterone to dihydrotestosterone. When compared to the occipital
scalp, the frontal scalp of young men with androgenic alopecia contains higher levels of dihydrotestosterone, a type 2 5α-reductase enzyme, and androgen receptors. The conversion and deposition of dihydrotestosterone from testosterone occur due to the action of an enzyme 5α-reductase, which is present at the pilosebaceous unit. After conversion to dihydrotestosterone, it binds to the androgen receptor, and this hormone-receptor complex activates the genes responsible for the shrinkage of hair follicles. Men with androgenic alopecia are found to have higher 5α-reductase, lower total testosterone, higher unbound/free testosterone, and higher free androgens, dihydrotestosterone. Cross talk occurs between androgens and the Wnt-beta-catenin signaling pathway that leads to hair loss. At the somatic stem cell level, androgens promote differentiation of facial hair dermal papillae but inhibit it at the scalp [1]. There are different stages in which hair loss takes place in the scalp as classified by Norwood.

2. Treatment options for treating androgenic alopecia

As hair loss is considered as a serious matter, many remedies have been evaluated to a great extent. Both males and females have issues regarding hair loss.

2.1. Hormonal treatment

The level of androgen plays an important role in the treatment of androgenic alopecia. The role of androgens in the etiology of androgenetic alopecia is considered to be a widespread aid in the treatment. Antiandrogens are usually delivered systemically in women and act by blocking the androgen receptors. They are contraindicated in men due to their feminizing action. Topical estrogens and antiestrogens have been used in both men and women [2].

2.2. Surgery

Surgery involving restoration of hair involves transplantation, scalp reduction surgery, or a combination of both. Hair transplantation is considered to be less invasive. Follicles that are not affected by miniaturization are redistributed over the scalp under local anesthesia. The result of hair transplantation is based on the texture of hair, the quality of hair, and also the number of transplanted hair in relation to the area to be covered or densified.

2.3. Combination therapies

A combination of medical and surgical therapy seems to be superior to surgery alone. A study revealed better clinical results for male patients treated with combination of finasteride 1 mg daily and hair surgery versus male patients treated with hair surgery alone, 12 months after follicular unit transplantation.
3. Application of drug delivery in the treatment of androgenic alopecia

The skin is a multilayered structure composed of stratum corneum, the outermost and the tightest layer of the skin and below which lies the epidermis and dermis. This highly hydrophobic layer is composed of differentiated nonnucleated cells, corneocytes, which are filled with keratins and embedded in the lipid domain. Since the rate-limiting step for the skin absorption of most molecules is due to this nonviable layer, percutaneous permeation of molecules is believed to be governed by diffusion laws. The skin contributes to 4% of the total body weight. The extent of skin permeation of a compound may depend on the route of absorption. Application of novel drug delivery systems to target and enhance permeation of drugs through and to the skin has been on the rise from the past decades. Topical route of delivery system is a noninvasive method that enables the drug to reach the systemic circulation. There are three pathways which are involved in the transdermal permeation of drugs: (1) through the intercellular lipid zone in the stratum corneum, (2) through the skin appendages, and (3) through the keratin bundles in the stratum corneum.

4. Ethosomes

In the recent years, application of vesicles for the delivery of drugs has gained attention owing to their amphiphilicity, biodegradability, nontoxic nature, and possibility to modulate the pharmacokinetic profile, and their applications in the coming years might further escalate. Further, their structural characteristics like size, shape, surface charge, and composition can be tailored as per the need. Vesicular systems 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. They can encapsulate both hydrophilic and lipophilic drugs and can release the drug in a sustained fashion.

The vesicular drug delivery systems are categorized based on the presence of lipid (conventional liposomes, deformable liposomes or transfersomes, ethosomes), polymer (polymersomes), surfactants (niosomes), and pharmacosomes, cubosomes, virosomes, and sphingosomes [3]. This chapter throws light on ethosomes.

Initially, because of interdigitation effect of ethanol on the bilayers, it was thought that the presence of ethanol would destruct liposomes. Thus, even if ethanol was used during the preparation of liposomes, it was removed [4]. But, Touitou et al. were able to show that the presence of ethanol did not show such effect and the existence of ethanolic vesicles has been confirmed by $^{31}$P nuclear magnetic resonance, dynamic light scattering, transmission electronic microscopy, and scanning electron microscopy. Ethosomes are soft, malleable lipid-based vesicular delivery systems consisting of amphipathic phospholipids arranged in one or more concentric bilayers enclosing numerous aqueous compartments (Figure 1) [5]. As the name suggests, ethosomes consist of ethanol (up to 45%) along with phospholipid and water.
Due to its amphiphilic nature, ethosomes can encapsulate and enhance the delivery of both hydrophilic and lipophilic drugs across the skin. When compared to the other vesicular drug delivery systems, ethosomes contain the highest amounts of ethanol (20–45%). In simpler terms, ethosomes can be described as liposomes with high ethanol content. The average size of ethosomes ranges between tens of nanometers and microns [4]. They can permeate across the skin dermal layers and deliver the drug to the deep skin or systemic circulation. The higher content of ethanol fluidizes both the ethosomal lipids and bilayers of the skin and facilitates permeation across the skin, thereby enhancing the availability of the drug to the blood.

4.1. Composition of ethosomes

The components of ethosomes are categorized under GRAS (generally recognized as safe) and usually contain ethanol, phospholipids, and water. Sometimes, they also contain propylene glycol, isopropyl alcohol, transcutol, and other surface modifiers to enhance the solubility of the drug(s) in the solvents and to enhance the permeation of drugs across the membranes. Some of the lipids which have been prominently used in the ethosomes are phosphatidylcholines and phosphatidylethanolamine containing compounds like Phospholipon 90, Soya phosphatidylcholine (S-75), and Lipoid S 100 [4].

Many experimental works have been carried out to study the effect of excipients on the size of the vesicles. It has been reported by Touitou et al. that as the amount of ethanol increases (20–45%), the size of the vesicles reduces (193–103 nm) and the amount of drug that permeates to and through the skin increases. An increase in the zeta potential to more negative values could be a possible reason for the decrease in the size of the ethosomes. They have also observed that up on increasing the amount of phospholipid (0.5–4%) the particle size of the ethosomes...
increased (118–249 nm). Dayan and Touitou have shown that the nature of drug could also play a role in determining the particle size. They have observed a reduction in particle size (154–90 nm) upon increasing the concentration of trihexyphenidyl HCl (0–3%), an anti-M1 muscarinic activity used for treating Parkinsonism [6]. The researchers have attributed the reduced particle size to the surface activity of the compound.

4.2. Preparation of ethosomes

Ethosomes can be prepared in a simple manner and usually does not require state of the equipments using a cold method. However, few methods like, hot method, thin-film hydration technique, and transmembrane pH gradient methods have also been reported for the preparation of ethosomes.

4.2.1. Cold method

This is the most commonly followed simplest method for preparing ethosomes and does not require any special equipment, and the process can be easily scaled up. Briefly, the lipid and drug will be dissolved in ethanol (or mixture of ethanol and glycols), followed by addition of sufficient amount of water as a slow stream with constant stirring for sometime. Proper care should be taken to prevent the evaporation of ethanol, and the temperature during the whole preparation should be maintained at 30°C [5].

4.2.2. Hot method

This method is similar to the cold method except for the fact that the ethanolic mixture is heated to 40°C [7].

4.2.3. Thin-film hydration technique

The lipids will be dissolved using organic solvent in a round bottom flask, and the organic solvent is evaporated above the lipid transition temperature using a rotary evaporator. The thin film formed around the inner walls of the round bottom flask will be hydrated using ethanolic mixture and dispersed with a probe sonicator to obtain a suspension of ethosomes [8].

4.3. Advantages

1. Ethosomes enhance permeation of drugs across/through the skin in an efficient manner, thereby enabling the drug to reach the desired site in the skin or to the blood.
2. Ethosomes can deliver both hydrophilic and lipophilic molecules, peptides, and other macromolecules.
3. Higher entrapment efficiencies of drugs when compared to liposomes can be observed.
4. The components of the ethosomes are generally recognized as safe (GRAS) and approved for pharmaceutical and cosmetic use.
5. Excellent stability over long periods can be observed.
6. Alcohol in the ethosomes acts as natural preservative, and hence there is no necessity to add any other preservatives.
7. There is no necessity of using high-end instruments for producing ethosomes, and large-scale production is feasible.
8. The cost of manufacturing ethosomes is very cheap.
9. Good patient compliance can be observed.
10. The transport of drugs across the skin is not concentration dependent.
11. It has numerous applications in pharmaceutical, veterinary, and cosmetic segments [4, 9, 10].

4.4. Disadvantages

1. Allergic reaction can be observed if the patients are allergic to ethanol or to any of the components of the ethosomes [4, 9, 10].
2. Ethosomal carriers are pertinent only for transdermal application unlike other carriers (solid lipid nanoparticles, polymeric nanoparticles, etc.) which can be used for multiple routes.
3. As ethanol is inflammable, proper care should be taken during preparation, application, transportation, and storage.

4.5. Mechanism of permeation across the skin barriers

Ethosomes are widely explored for the delivery of the drugs across the skin. The fluidizing effect of ethanol on the lipid bilayers of the stratum corneum together with the softness of the ethosomal carrier gives them the capability to penetrate the perturbed SC lamellae more easily, thus promoting delivery of the actives into the deep layers of the skin and through the skin.

Touitou et al. proposed a hypothetical action mechanism of ethosomal system for its enhanced permeation across the skin. First, ethanol interacts and disturbs the organization of the stratum corneum lipid bilayer and enhances its lipid fluidity by reducing the melting point. The flexible ethosome vesicles can then penetrate the disturbed stratum corneum bilayers. During the process of penetration, these ethosomes fuse with the lipids present in the skin along its pathways and promote release of the drug at various points. When compared to other vesicles, occlusion slightly increases the penetration of ethosomes across the skin, which indicates that an osmotic gradient across the skin is not necessary. These data differ from that observed with elastic vesicles where permeation enhancement occurred only in nonocclusive conditions and points toward different mechanisms of action of the carriers [4, 9].

4.6. Applications of ethosomes

Ethosomes were found to highly enhance the permeation across the skin when tested against individual components of the system (ethanol, hydroethanolic solution, and ethanolic
More research has been concentrating on its application across the skin. Some of the applications of ethosomes are [3, 7, 9, 11–13]:

1. Ethosomes can be used as an efficient drug delivery system for the delivery of antibiotics to the bacteria localized within the deep skin strata to eradicate staphylococcal infections.

2. Ethosomal carrier systems can be used as a carrier for treating various dermal-based inflammatory infections.

3. Ethosomes can be used for the topical delivery of anti-psoriatic drugs.

4. It can be used for targeted delivery of drugs to the pilosebaceous units and hair follicles.

5. Ethosomes can be used for androgenic deficiency problems related to menopausal and postmenopausal syndromes.

6. Erectile dysfunction problems can also be treated using ethosomes.

4.7. Characterization of ethosomes

After preparing the ethosomes, it is highly necessary to characterize them on the basis of particle size, morphology, zeta potential, entrapment efficiency, physical state of the entrapped drug, and permeation studies across the skin.

The particle size can be measured by dynamic light scattering or photon correlation spectroscopy using Malvern particle size analyzer. Dynamic light scattering measures the average hydrodynamic diameter of particles by measuring the changes in the speckled pattern produced by the scattered light of the particles in Brownian motion. Additionally, the surface charge of the ethosomes can also be measured using Malvern particle size analyzer using a suitable probe (zeta dip cell). The zeta potential provides information about the stability of the ethosomes during storage. The morphology of the ethosomes can be visualized by atomic force microscope and transmission electron microscope. Using transmission electron microscope, 1D, 2D, and 3D imaging, measuring, modeling, and manipulating matter can be accomplished. It is based on the absorption of electron beam as it passes through ultrathin (<100 nm) samples. The AFM utilizes piezoelectric ceramics to move a specimen in nanoscale increments in the X, Y, and Z directions. The basic principle of atomic force microscope depends on the measurement of the interactive force between a tip and the sample surface using special probes made by an elastic cantilever with a sharp tip on the end.

The amount of drug entrapped in the ethosomes can be measured by separating the ethosomes from the free drug. By centrifuging the sample at high speed, heavier ethosomes form sediment at the bottom by leaving out the free drug in the supernatant. Estimating the free drug would give an idea on the amount of drug entrapped in the ethosomes. Using differential scanning calorimetry, the transition temperature of the vesicular lipid systems and the physical state of the entrapped drug can be identified.Using excised skin, the rate and the ability of the ethosomes to permeate the skin can be measured. Additionally, the mechanism by which the ethosomes permeate the skin can also be visualized by confocal laser scanning microscope.
4.8. Stability of ethosomes

The presence of ethanol and phospholipids induces high negative charge and can allow the formulations to remain stable over a long period of time. A number of studies have shown that ethosomes of trihexyphenidyl HCl were stable for at least 2 years. In another study, erythromycin ethosomes were found to be stable for 1 year. Even a marketed ethosomal formulation of acyclovir (Supra-Vir cream, Trima, Israel) has shown decent stability for 3 years. Finasteride-loaded ethosomes prepared in our laboratory have shown excellent stability for over 2 years. Hence, ethosomes offer a good stability as a drug carrier for a long period.

4.9. Safety of ethosomes

In vitro cell line studies carried out for ethosomes on skin cells (three T3 fibroblasts) did not show any toxicity. Histological observations also did not show any marked changes in the structure and thickness of the horny layer, and inflammatory cells were not observed. Both acute and continuous applications of ethosomal patch for 2 weeks in rabbits did not produce any skin irritation. Estimation of biochemical parameters in rat’s blood (with regard to liver, kidney, and muscle function parameters) after 5 days of treatment with transdermal ethosomal ibuprofen gel also did not show significant differences among the treated group and the control group. Even skin tolerability tests by reflectance spectrophotometry using ethosomal systems on healthy human subjects did not induce skin erythema 12, 24, and 48 h after application. But, application of hydroethanolic solution using the same drug with an equal water/ethanol ratio to that of ethosomes resulted in significant skin erythema. Furthermore, application of various ethosomes (clindamycin and salicylic acid, acyclovir, or PGE1) to the skin of human volunteers in three clinical studies did not show any adverse skin reactions. Moreover, products formulated with ethosomal carriers have been in use for a number of years, without any reports on skin irritation or safety issues [9].

5. Book to bench experience on application of ethosomes for androgenic alopecia

5.1. Application of finasteride-loaded ethosomes

This section has been added to share our first-hand experience on developing ethosomes of finasteride in treating androgenic alopecia. As stated earlier, androgenetic alopecia is caused by the deposition of dihydrotestosterone at the androgen receptors present in the pilosebaceous unit. Finasteride is a potent 5-α reductase inhibitor which can prevent the conversion of testosterone to dihydrotestosterone. The conversion and deposition of dihydrotestosterone from testosterone occur due to the action of an enzyme 5-α reductase, which is present at the pilosebaceous unit. After conversion to dihydrotestosterone, it binds to the androgen receptor, and this hormone-receptor complex activates the genes responsible for the shrinkage of hair follicles. Hence, targeting specifically 5-α reductase
present at the pilosebaceous unit can be a good option for treating androgenetic alopecia. Although finasteride is a clinically proven and FDA-approved type 2 5α reductase inhibitor, which can potentially inhibit the conversion of testosterone to dihydrotestosterone, its oral administration in males causes infertility. Thus, we have developed ethosomes of finasteride using a cold method (Figure 2) to target the pilosebaceous unit. Further, we have evaluated the ability of different permeation enhancers to enhance permeation across human frontal scalp skin and rat skin using Franz diffusion cell (Figure 3). The particle size of various formulations was found to be in the range of 105–227 nm (Figure 4). A maximum entrapment efficiency of ~90% was observed for this lipophilic drug. These ethosomes permeated well across both human frontal scalp skin and rat skin when compared to finasteride solubilized in pH 7.4 phosphate buffer saline. The values of transdermal flux across the skin showed that permeation enhancers (oleic acid, thymol, and isopropyl myristate) enhanced the permeation of finasteride across the skin. The most highlighting fact is its stability for 4 years in refrigerated conditions. We did not observe any increase in size during this period possibly due to the stabilization of the particles in the ethanolic medium [14]. Hence, ethosomes represent an exciting and the most promising drug delivery system for treating androgenic alopecia. As the preparation method is simple, ethosomes can also have good industrial viability, and we expect several ethosomal products to be marketed in the upcoming years.

![Preparation of ethosomes](Figure 2. Preparation of ethosomes.)
Through this research work, an attempt was made to prepare ethosomes containing herbal extracts of *Phyllanthus niruri*, *Croton tiglium* and *Zingiber officinale*. In ancient Indian Ayurvedic scripts, these plants have been reported to combat androgenic alopecia. Initially, ethanolic extracts of *Phyllanthus niruri* (aerial parts), *Croton tiglium* (seeds), and *Zingiber officinale* (rhizomes) were prepared. After screening these extracts for preliminary constituents, the active components were identified using high-performance thin-layer chromatography and gas chromatography mass spectroscopic analysis. Ethosomes containing these herbal extracts were
prepared using cold method. The particle size range of the ethosome formulations were in the range from 167 to 1524 nm. The histopathology of the rat skin after applying these ethosomes on the skin of rats did not show any changes in the morphology (Figure 5). Additionally, these ethosomes did not show any toxicity toward immortalized human keratinocytes (HaCaT) cell line. Currently, we are testing the effectiveness of these formulations in an animal model for their ability to treat androgenic alopecia (Figure 6).

6. Conclusion

Ethosomes have shown a tremendous promise in their ability to permeate skin. They can be safely used to deliver the drugs to and through the skin. Ethosomes can be an excellent potential carrier for the transportation of drugs to the pilosebaceous unit of the scalp, the main site for the deposition of DHT, for treating androgenic alopecia.
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Conflict of interest

There is not conflict of interest among the authors.

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