Available online at www.pharmacie-globale.info ISSN 0976-8157

Review Article

PHARMACIE GLOBALE

INTERNATIONAL JOURNAL OF COMPREHENSIVE PHARMACY

ETHOSOMES: A NOVEL DRUG CARRIER

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Received: 22 March 2011; Revised: 25 June 2011; Accepted: 30 June 2011; Available online: 5 July 2011

ABSTRACT

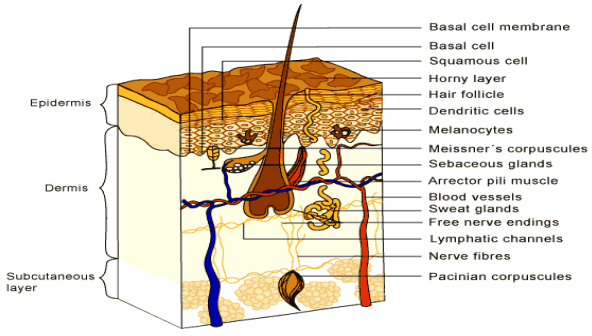
Skin acts as a major target as well as a principal barrier for topical/transdermal drug delivery. Despite the many advantages of this system, the major obstacle is the low diffusion rate of drugs across the stratum corneum. Several methods have been tried to increase the permeation rate of drugs temporarily. One simple and convenient approach is application of drugs in formulation with elastic vesicles or skin enhancers. Vesicular system is one of the most controversial methods for transdermal delivery of active substances in that ethosome are the ethanolic phospholipids vesicles which are used mainly for transdermal delivery of drugs. Ethosomes have higher penetration rate through skin due to its ethanolic content. In this article reviews various aspect of ethosomes including their mechanism of penetration, preparation, advantages, characterization, composition, preparation, application and marketed product. These carriers open new challenges and opportunities for the development of novel improved therapies.

Keywords: Ethosomes, transdermal, vesicular carriers, ethanol, phospholipid.

INTRODUCTION

Skin forms a protecting covering layer against the external environment and prevents water loss from the underlying tissue. It is flexible enough to resist permanent distortion from movement and thin enough to allow the perception of stimuli. It also performs many ancillary functions such as synthesis and metabolism and the production of sweat enables temperature control and excretion of waste products by means of sweating etc.[1,2] It has been also reported that skin protects the body from antigenic stimuli by means of a part of the immune system known as skin associated lymphoid tissue.[3] The skin can be considered to be composed of three layers: subcutaneous tissue, dermis and epidermis layer[4] as shown in figure **1**.

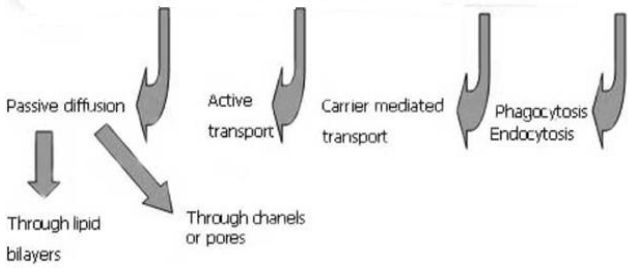
Figure 1. Structure of skin



Stratum corneum is the outermost layer of the epidermis. It consists of 10 to 25 layers of dead, elongated, fully keratinized corneocytes, which are embedded in a matrix of lipid bilayers.[5,6] It has been shown that the stratum corneum is the main barrier to penetration through the skin. When a topical formulation is placed on the skin, the active drug is required to penetrate through the stratum corneum into the viable tissue. The limiting factor for these processes is the slow diffusion through the dead horny layer of skin.[7-10] Stratum corneum behaves as a hydrophobic membrane. The rates of permeation of skin by low and high molecular weight organic non-electrolytes are mostly determined within the stratum corneum.[11,12]

The molecular structures and appearance of the molecules can be examined using molecular modeling computer programs. There have been many discussions on the route of penetration as shown in figure **2**.

Figure 2. Main routes of penetration



Under normal conditions, the main route is observed through the intercellular spaces or lipid bilayers.[13,14] The diffusional path length is therefore much longer than simple thickness of the stratum corneum (20-30 mm). The penetration through skin is also affected by several biological factors such as skin age, body site, skin condition and diseases, water content of the skin or hydration. The intercellular spaces contain structured lipids/proteins and a diffusing molecule has to cross a variety of lipophilic and hydrophilic domains before reaching to the stratum corneum and viable epidermis junction. Although the nature of the barrier is very heterogeneous, the diffusion through the skin can be described by simple Fick's laws.[15]

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To overcome the stratum corneum barrier, various mechanisms have been investigated, including use of chemical or physical enhancers such as iontophoresis, sonophoresis, etc. Liposomes, niosomes, transferosomes and ethosomes also have the potential of overcoming the skin barrier and have been reported to enhance permeability of drug through the stratum corneum barrier. The non-invasive approaches for providing transdermal drug delivery of various therapeutic substances are:

Drug and vehicle interactions
- Selection of correct drug or prodrug
- Chemical potential adjustment
- Ion pairs and complex coacervates
- Eutectic systems

Stratum corneum modification
- Hydration
- Chemical penetration enhancers

Stratum corneum bypassed or removed
- Microneedle array
- Stratum corneum ablated
- Follicular delivery

Electrically assisted methods
- Ultrasound (Phonophoresis, Sonophoresis)
- Iontophoresis
- Electroporation
- Magnetophoresis
- Photomechanical wave

Vesicles and particles
- Liposomes and other vesicles
- Niosomes
- Transfersomes
- ethosomes

VESICULAR APPROACHES FOR TOPICAL DRUG DELIVERY

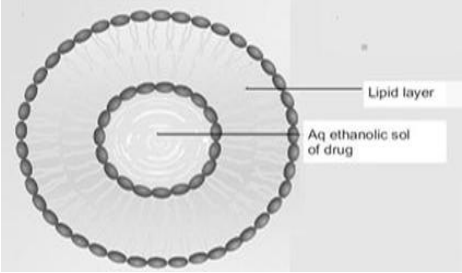
Drug encapsulated in lipid vesicles prepared from phospholipids and nonionic surfactants is known to-be transported into and across the skin. Lipids present in the skin contribute to the barrier properties of skin and prevent systemic absorption of drugs. Due to the amphiphilic nature, lipid vesicles may serve as non-toxic penetration enhancer for drugs. In addition, vesicles can be used for encapsulating hydrophilic and lipophilic as well as low and high molecular weight drugs. Therefore, these lipid rich vesicles are hypothesized to carry significant quantity of drugs across the skin thus, enhancing the systemic absorption of drugs.

Drug delivery from liposomes in transdermal formulation has been studied for many purposes but unstable nature and poor skin permeation limits their use for topical delivery. In order to increase the stability of liposomes, the concept of proliposomes was proposed. This approach was extended to niosomes, which exhibited superior stability as compared to liposomes . However, due to poor skin permeability, liposomes and niosomes could not be successfully used for systemic drug delivery and their use was limited for topical use. To overcome problems of poor skin permeability Cevc et al. and Touitou et al. recently introduced two new vesicular carrier systems transfersomes and ethosomes, respectively for non-invasive delivery of drugs into or across the skin. Transfersomes¨ and ethosomes incorporated edge activators (surfactants) and penetration enhancers (alcohols and polyols), respectively, to influence the properties of vesicles and stratum corneum. The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Researchers have understood the properties of vesicles structure for use in better drug delivery within their cavities, which would to tag the vesicle for cell specificity. One of the major advances in vesicle research was the finding a vesicle derivatives, known as an Ethosomes.[16]

ETHOSOMES AS A NOVEL CARRIER

Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are composed mainly of phospholipids, (phosphatidylcholine, phosphatidylserine, phosphatitidic acid), high concentration of ethanol and water as shown in figure **3**. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization; therefore, when integrated into a vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. Also, because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improves drug distribution ability in stratum corneum lipids.

Figure 3. Structure of Ethosomes



Ethosomes are soft, malleable vesicles composed mainly of phospholipids, ethanol (relatively high concentration) and water. These “soft vesicles” represents novel vesicular carrier for enhanced delivery to/through skin. The size of Ethosomes vesicles can be modulated from tens of nanometers to microns.[17]

MECHANISM OF PENETRATION

The enhanced delivery of actives using ethosomes over liposomes can be ascribed to an interaction between ethosomes and skin lipids. A possible mechanism for this interaction has been proposed. It is thought that the first part of the mechanism is due to the ‘ethanol effect whereby intercalation of the ethanol into intercellular lipids increasing lipid fluidity and decreases the density of the lipid multilayer.

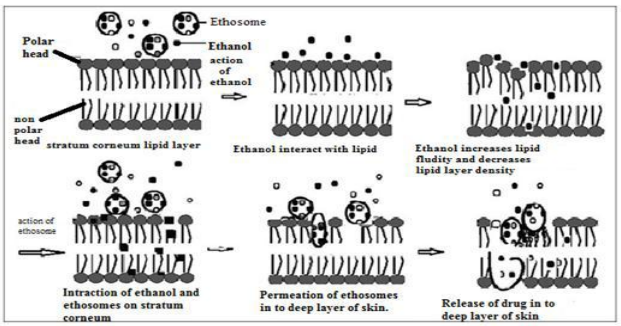
This is followed by the ‘ethosome effect’, which includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug in deep layers of the skin as shown in Figure 4. The drug absorption probably occurs in following two phases:

Ethanol effect: Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhancing effect is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

Ethosomes effect: Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. So the ethosomes permeates very easily

inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin.[17]

Figure 4. Mechanism of penetration



Advantages of ethosomal drug delivery

- Ethosomes are enhanced permeation of drug through skin for transdermal and dermal delivery.
- Ethosomes are platform for the delivery of large and diverse group of drugs (peptides, protein molecules)
- Ethosome components are approved for pharmaceutical and cosmetic use.
- Low risk profile- The technology has no large-scale drug development risk since the toxicological profiles of the ethosomal components are well documented in the scientific literature.
- High patient compliance- The ethosomal drug is administrated in semisolid form (gel or cream), producing high patient compliance by is high. In contrast, iontophoresis and phonophoresis are relatively complicated to use which will affect patient compliance.
- High market attractiveness for products with proprietary technology. Relatively simple to manufacture with no complicated technical investments required for production of Ethosomes.
- The ethosomal system is passive, non-invasive and is available for immediate commercialization.
- Various applications in Pharmaceutical, Veterinary, Cosmetic field.

Characterization of ehosomes

- **Vesicle shape:** Ethosomes can be easily visualized by using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM).[18]
- **Size and zeta potential:** Particle size of the ethosomes can be determined by dynamic light scattering (DLS) and photon correlation spectroscopy (PCS). Zeta potential of the formulation can be measured by Zeta meter.[19]
- **Transition temperature:** The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry (DSC).[20]
- **Drug entrapment:** The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique.[21]
- **Drug content:** Drug content of the ethosomes can be determined using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method.[22]
- **Surface tension measurement:** The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.
- **Stability studies:** The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. Mean size is measured by DLS and structure changes are observed by TEM.
- **Skin permeation studies:** The ability of the ethosomal preparation to penetrate into the skin layers can be determined by using confocal laser scanning microscopy (CLSM).[23]

Composition of ethosomes[24]

Table 1. Different additives employed In formulation of ethosomes

Material	Examples	Uses
Phospholipid	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline Distearyl phosphatidyl choline	Vesicles forming component
Polyglycol	Propylene glycol Transcutol RTM	As a skin penetration enhancer
Alcohol	Ethanol Isopropyl alcohol	For providing the softness for vesicle membrane As a penetration enhancer
Cholesterol	Cholesterol	For providing the stability to vesicle membrane
Dye	Rhodamine-123 Rhodamine red Fluorescen Isothiocynate (FITC) 6- Carboxy fluorescence	For characterization study
Vehicle	Carbopol 934	As a gel former

Methods for preparation

Cold Method: This is the most common method utilized for the preparation of ethosomal formulation. In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer. Propylene glycol or other polyol is added duringstirring. This mixture is heated to 30°C in a water bath. The water heated to 30°C in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication or extrusion method. Finally, the formulation is stored under refrigeration.[25-27]

Hot method: In this method phospholipid is dispersed in water by heating in a water bath at 40°C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40°C. Once both mixtures reach 40°C, the organic phase is added to the aqueous one.

The drug is dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method.[28]

Applications

Ethosomes are used in pilosabeceous targeting. Ethosomes, the high ethanol containing vesicles are able to penetrate the deeper layers of the skin and hence appear to be vesicles of choice for transdermal drug delivery of hydrophilic and impermeable drugs through the skin.

CONCLUSION

Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies Transdermal route is promising alternative to

drug de-livery for systemic effect. Ethosomes has initiated a new area in vesicular research for transdermal drug delivery which can provide better skin permeation than liposomes. The main limiting factor of transdermal drug delivery system i.e. epidermal barrier can be overcome by ethosomes to significant extent. Application of ethosomes provides the advantages such as improved permeation through skin and targeting to deeper skin layers for various skin diseases. Ethosomes have been tested to encapsulate hydrophilic drugs, cationic drugs, proteins and peptides. Further, research in this area will allow better control over drug release in vivo and long-term safety data, allowing the therapy more effective. Thus, ethosomal formulations possess promising future in effective dermal/transdermal deli-very of bioactive agents.

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