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Review

Advances in studies of phospholipids as carriers in skin topical application

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Abstract

Objective: This article provides an overview of characteristics of phospholipids, the characteristics and influential factors of liposome and microemulsion as carriers for skin delivery of drugs, and the latest advances of the phospholipids carriers in transdermal delivery systems. The perspective is that phospholipids carriers may be capable of a wide range of applications in the transdermal delivery system.

Key words: phospholipids liposome; phospholipids microemulsion; skin topical application; drug carriers

INTRODUCTION

Phospholipids share a high structural similarity with skin lipids and thus have many advantages such as strong tissue affinity, biodegradability and very low toxicity, which have promoted their increasingly wider applications in transdermal delivery systems. In order to facilitate discussion on phospholipids, this review provides an overview of the latest research progress on the application of phospholipids drugs carriers in transdermal delivery, and also includes the characteristics of phospholipids, and recent research advances on the application of liposomes and phospholipid microemulsion as dermal and transdermal drug carriers. The characteristics and influential factors of liposome and microemusion as carriers for skin delivery of drugs are also studied in order to offer a valuable reference for further development of these types of drugs.

Characteristics of phospholipids as transdermal drug delivery carriers

Stratum corneum is composed of keratinocytes and intercorneocyte matrix. In Keratinocytes, cellular membranes account for 50% of the dry mass and consists mainly of phosphatidyl choline and sphingomyelin. The intercorneocyte matrix is rich in phospholipids. Thus, phospholipids are the main components of stratum corneum.

Phospholipids(being biological in origin) are not immunogenic and are widely found in all animals and plants in nature, such as soya oil, rape seed oil, corn oil, yolk, liver, brain and marrow. Thus, they are nonirritant and harmless to skin.

Their hydrophilicity and lipophilicity enable them to act as carriers for hydrophilic and lipophilic drugs, as well as drugs that are hard to dissolve. Therefore they can effectively transport drugs to the target cells for release, allowing the drug to take effect as intended. Thus, they can serve as a good carrier for skin targeted drugs.

Phospholipids as various transdermal delivery carriers

Liposome

(1)Characteristics of liposome

Liposomes are small spherical vesicles composed of one or multiple layers of phospholipid bilayer mem-

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branes. Liposomes are amphiphilic(both hydrophilic and hydrophobic) so effectively encapsulates both hydrophilic and lipophilic drugs. The characteristics of liposomes as carriers of skin topical application are as follows.

(a)Effective skin targeting

Liposome enhances drug penetration through the stratum corneum and deposits in the epidermis as well as dermis, while reducing systemic absorption. Therefore liposome is a very suitable drug carrier for diseases of the skin, especially the epidermis.

(b)Increasing drug solubilization

Because of its amphipathicity, liposome can encapsule hydrosoluble as well as liposoluble drugs. For some hard-to-dissolve drugs, liposomal encapsulation can enhance their solubility and increase their transdermal penetration.

(c)Delayed release of drugs

Liposmes can form deposits for drugs in the epidermis and dermis and release drugs to diseased cells directly and for a prolonged duration. Therefore, liposomal preparations are especially suitable for chronic and relapsing skin diseases.

(d)Low toxicity to skin

The components of liposome are usually intrinsic components of organisms, so liposomes are of little toxicity and are biodegradable. They are also not an irritant to skin.

(2)Drug absorption influence factors for liposome as a carrier for skin topical applications.

(a)Composition of liposomes:Coderch et al[1] investigated the influence of liposome composition on percutaneous penetration using electron paramagnetic resonance(EPR), and demonstrated that phospholipid composition(hydrogenated or not hydrogenated) and the amount of cholesterol have very significant influence on the penetration behavior of liposomes, which is closely related to membrane fluidity.

(b)Size of liposomes:Vermathe et al[2] investigated the influence of liposome size on the skin penetration of liposomes encapsulating a hydrophilic fluorescent compound(CF) or a lipophilic compound(DiL) using Franz diffusion cell and confocal microscopy. The study indicated that for the hydrophilic compound CF, at the liposomes size of 120 nm(in diameter), the highest deposition of CF was found in the SC, skin layers, and receiptor medium. The concentration of CF carried by the 120 nm liposomes, compared with liposomes of 91 nm, 377 nm, and 810 nm, was respectively 1.12, 1.19, 1.83 fold high in SC, 4.68, 7.92, 33.57 fold in skin, and 1.52, 2.38, 7.11 fold in the receiptor medium. For the lipophilic compound DiL, the highest fluorescence was found in SC, skin and receptor medium at liposome size of 71 nm, indicating large liposomes are not effective in penetrating skin.

(c)Surface charge of liposomes: Ogisot et al[3] revealed that the *in vitro* penetration rate of melatonin(MT) entrapped in negatively charged liposomes was higher than that of positively charged ones, and concluded that the negative charge improves skin permeation of liposomes. Sinico et al[4] demonstrated by high pressure liquid chromatography(HPLC) that negatively charged liposomes markedly improves tretinoin(TRA) retention in the skin of newborn pigs.

(d)Zeta electric potential and pH value:Zeta electric potential mainly influences liposome stability. Zeta potential exceeding 60 mV indicates high liposome stability, 40-60 mV considerable stability, 30-40 mV moderate stability, and 10-30 mV instability of liposomes. The pH value influences phase-transition temperature, a parameter of transdermal penetration of liposomes. Thus, different pH values will certainly influence the transdermal penetration of liposomes.

In view of the merits of liposomes in skin topical application and their influencing factors, the parameters of liposomal preparations for skin application could be achieved by adjusting liposomal composition, particle size, surface charge, electric potential and pH. Compared with other external skin preparations, such as creams and liniments, liposomes provide more adjustable parameters in their preparation, and in treatments offer the advantages of enhancing drug effects, shortening the expected treatment course and lowering side effects.

(3)Latest laboratory and clinical progress in skin topical application of liposomes

Liposomal drugs in skin topical application can be categorized as steroid hormones, anti-inflammatory agents, and functional cosmetics, the latter have been an increasing trend in recent years. On the other hand, studies on liposome itself have been continuously deepened in order to make liposomes better drug carriers, such as enthosomes, deformable liposomes and so on.

(a) Conventional liposomes:Shigeta et al[5] demonstrated, through UV-stimulation causing dorsal skin hyperpigmentation in brownish guinea pigs, that the whitening effect of hydrogel containing liposomal linoleic acid(LA) was far greater than hydrogel containing free LA in ethanol. In UV-stimulated hyperpigmented human upper arm skin, they demonstrated that liposomal LA(0.1%) resulted in a whitening effect comparable to 10.0% non-liposomal LA, and far greater than 3.0% non-liposomal LA. These results indicate that liposomal formulations are favorable for the transdermal application of LA. Abeer et al[6] modified oral cetirizine to topical application in

liposomal formulation and assessed post-application peripheral H1-antihistaminic activity and extent of systemic absorption in a model of rabbit back skin histamine-induced wheal formation test. The results indicated that cetirizine formulated in the multilamellar vesicle(MLV) was faster in taking effect, acted longer and resulted in a lower plasma cetirizine concentration than cetirizine formulated in small unilamellar vesicles (SUV).

(b)Ethosomes:The main difference between conventional liposomes and ethosomes is that ethanol, instead of cholesterol, is encapsulated in ethosomes. Phospholipids are the main components in ethosomes, especially phosphatidyl choline(also known as lecithin). Paolino et al[7] evaluated percutaneous permeation of ammonium glycyrrhizinate(an effective drug for skin inflammation) in ethosomes by using Franz's cells. Ethosomes formulation, compared with water and ethnol solutions, led to an increase of *in vitro* percutaneous permeation of both methylnicotinate and ammonium glycyrrhizinate and enhanced the anti-inflammatory activity of ammonium glycyrrhizinate. In human volunteers the ethosomes suspension showed excellent skin tolerability for as long as 48 h application. Godin et al[8] investigated permeation of fluorescently labeled bacitracin through human cadaver and rat skin, and demonstrated that bacitracin in ethosome was delivered into deep layers of skin through SC intercorneocyte lipids. The efficient delivery of antibiotics to deep skin layers by ethosomal applications is highly beneficial since it helps to reduce possible side effects and other drawbacks associated with systemic treatment. Furthermore, ethosomal delivery systems could be used for the treatment of many dermal infections, (which is related to its capability to transit the SC and cellular membrane).

(c)Deformable liposomes: Deformable liposomes are characterized by at least one order of magnitude higher elasticity than conventional lipid vesicles and they are sufficiently deformable to penetrate pores 1/5 of their own size. In addition,because of their high hydrophilicity, they can permeate skin via transepidermal hydration gradient.

Oh et al[9] compared deformable liposomes with conventional neutral or negatively-charged liposomes and found deformable liposomes containing Tween-20 could significantly increase skin permeation of retinol, indicating potential applications of deformable liposomes in the formulation of retinol and other lipophilic functional cosmetic compounds. Gregor et al[10] studied transdermal delivery of hydrocortisone and dexmethasone with highly deformable liposomal carriers and found that the minimum effective dose was lowered by three to 5 times and the suppression of the drug-induced oedema was prolonged nearly 2-fold by the deformable liposomes compared with the cr è me or lotion-based products. The minimum effective drug dose of dexamethasone was lowered more than 10 times with highly deformable vesicles and suppression of the drug-induced oedema was prolonged by nearly 4-fold. The results indicated that topical corticosteroid delivery with highly deformable liposome vesicles could improve therapeutic risk-benefit ratio by decreasing drug dose and improving drug stability, which is related to better targeting and longer drug presence in the skin brought by deformable liposomes.

Deformable liposomes were also reported to improve skin permeation and deposition of cyclosporin A, methotrexate, and melatonin[11-13]. On the other hand, deformable liposomes were reported to significantly improve ketotifen skin delivery with greater improvement of skin deposition than improvement of skin permeation, suggesting its role being more useful for dermal than for transdermal delivery of ketotifen[14]. Similarly, deformable liposomes were reported to improve only skin deposition instead of skin permeation of 5-fluorouracil and dipotassium glycyrrhizinate[15-16], and hence were considered only useful for dermal delivery of these drugs.

Phospholipids microemulsions

(1)Characteristics of phospholipids microemulsion

Microemulsion is usually composed of an aqueous phase, oil phase, a surfactant and co-surfactant. By structure, it can be classified to water-in-oil microemulsion, oil-in-water microemulsion and water-in-oil-in-water double microemulsion. Compared with liposomes(which have the disadvantages of low encapsulation ratio and high tendency of phospholipid oxidization) phospholipids microemulsions as carrier vesicles in skin topical application have their unique characteristics:

(a)Stable structure: Microemulsions are thermodynamically stable, isotropic, oil and water mixture system. Their structure can not be destroyed even by high speed centrifugation.

(b)Small particle diameter: usually within the range of 10-100 nm.

(c)Phospholipids as surfactant:Phospholipids are components of biological membrane and have been shown to promote drug permeation in different modes. l-alpha-phosphatidylcholine from egg yolk and dioleylphosphatidyl ethanolamine from soybean, which are in a fluid state, diffuses into the stratum corneum and enhances dermal and transdermal drug penetration, while distearoylphosphatidyl choline, which is in a gel-state, has no such capability[17]. Phospholipids pose no

toxicity or irritation to skin at even high concentrations. Therefore, the use of phospholipids as a surfactant, reduces toxicity and side effects and improves drug permeation.

(d)Microemulsions can form automatically, without heating, by a simple process and thus, are suitable for transdermal delivery of protein and peptide drugs.

The above mentioned skin permeation characteristics of microemulsions attracted more and more basic and applied research on their use as carriers for the transdermal drug delivery system.

(2)Drug absorption influencing factors of microemulsion carriers for skin topical application.

(a)Water phase:The ratio of water phase in microemulsion is directly associated with transdermal permeation of microemulsion. When the ratio of water phase is not less than 87.5%, the particles are well-distributed and very stable[18].

(b)Oil phase:The ratio of water phase in microemulsion is directly associated with transdermal permeation of microemulsion. Saturated and unsaturated fatty acids are frequently used as oil phase as they are penetration enhancers. The most universally recognized being oleic acid[17].

(c)Cosurfactant:The ratio of surfactant/cosurfactant has a large influence on percutaneous permeation of microemulsion. Cosurfactant inserts itself into the interphase film consisting of surfactant to form a compound cohesion film, which improves fastness and compliance of the film, and increases the solubility of the surfactant. This will decrease the stability of the microemulsion to form smaller microemulsion drops, which will increase the percutaneous permeability of microemulsions.

(d)Drug concentration gradient in skin: The more drugs loaded in microemulsions, the higher penetration gradient is, and the better the penetration effect will be. Peira *et al*[19] studied the rate of transdermal penetration of apomorphine from microemulsions(with lecithin as surfactant and using skin of hairless mouse) and found increasing drug solubility in microemulsion increased the concentration gradient between microemulsion and skin and drug entered skin at an obviously higher rate.

(e)Others:Some additives can also enhance the penetration of microemulsions. Trotta et al[20]experimented with polydimethylsiloxane(PDMS) membranes and found decreased permeability of retinoic acid(RA) from lecithin-loaded o/w microemulsions in the presence of counter ions. In pig skin experiments, the permeation of RA microemulsions was much lower than RA solution, and skin deposition of RA o/w microemulsions was markedly increased in the presence of counter ions, which were believed to be related with the increased lipophilicity of the drug and the affinity of the counter ion to vehicle membrane. The results suggest that o/w microemulsions containing a counter ion could be used to optimize drug targeting and concomitantly decrease systemic absorption.

(3)Latest laboratory and clinical progress of phospholipids microemulsions in skin topical application.

(a)As carriers of drugs against skin diseases

Paolino *et al* [21] found that o/w microemulsions prepared with triglyceride, lecithin and ketoprofen showed a good human skin tolerability and higher skin permeation rate than conventional formulations, i.e. regular emulsion and Carbomer gel. The reason could be due to relatively higher drug concentration in microemulsion, penetration enhancing effect of lecithin, smaller size of microemulsion vesicles and larger skin contact areas.

(b)As carriers of gene vaccines

Microemulsions could serve as a method for transfection of dendritic cells by plasmid DNA(pDNA) and open a new way for protein or gene vaccine inoculation. Cui et al[22] prepared pDNA coated microemulsion nanoparticles directly from warm o/w microemulsion, with or without DOPE, and delivered them to dendritic cells in the shoulder skin of Balb/c mice. All pDNA-coated nanoparticles, especially the mannan-coated pDNA-nanoparticles with DOPE, resulted in significant enhancement in both antigen-specific IgG titers(16-fold) and splenocyte proliferation.

(c)As carriers of functional cosmetics

The excellent microemulsion deposition of drugs in stratum corneum and dermis lead to increasing studies of microemulsions application in cosmetics such as beauty care and sun products. Gallarate et al[23] used o/w microemulsion, with lecithin as mild, non-irritant surfactants, as a cosmetic vehicle for whitening agents arbutin and kojic acid, which showed higher stability under UVB irradiation in microemulsion than in aqueous solutions. For water-soluble sunscreen agents, which are frequently washed away from skin in aqueous solution, using o/w microemulsions as carriers renders them waterproof, non-sticky, and easy to spread[24].

Perspective

Phospholipids as drug carriers have some unique advantages which other conventional external preparations don't have. Recently studies about phospholipids as carriers for dermal and transdermal drug delivery indicated their wide application perspective which will be explored further by future studies.

Many have hoped that drug preparations could be made if considerations of the characteristics of phospholipids as carriers and the characteristics of distinctive skin diseases are taken into account, so as to ac-

tively target the diseased skin tissue with active drugs better deposited in epidermis and/or dermis and released in a prolonged time, which will improve and prolong the therapeutic efficacy of the drug, and lower the side effects. This type of application is particularly suitable for certain chronic and relapsing skin diseases, such as chronic eczema, psoriasis, neurodermitis, etc. The near future may witness the emergence of new commercial phospholipids-based topical products such as the Transfersome^{R} carrier series.

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